

UNIVERSITE DE GENEVE
Département de biologie animale

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THE RHYTHMICALLY ACTIVE METACARPAL BAT VEIN
(PTEROPUS GIGANTEUS) AS A MODEL FOR THE STUDY
OF VENOUS SMOOTH MUSCLE FUNCTION
AND PERIPHERAL AUTOREGULATORY FLOW MECHANISMS *

THESE

présentée à la Faculté des sciences de l'Université de Genève
pour obtenir le grade de docteur ès sciences biologiques

par

John PERISTIANY

de

Grèce

Thèse N° 1622

GENEVE
Editions Médecine et Hygiène

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La Faculté des sciences, sur le préavis du professeur H. Huggel, directeur de thèse, du professeur P. Moret, Faculté de médecine, du professeur d'histologie Ch. Owman, Université de Lund, Suède et du docteur Krähenbuhl (Policlinique de médecine), autorise l'impression de la présente thèse, sans exprimer d'opinion sur les propositions qui y sont énoncées.

Genève, le 5 juillet 1973

Le doyen:
Jean Muller

Thèse No 1622



LA VEINE METACARPIENNE PULSATILE DE LA CHAUVÉ-SOURIS
(PTEROPUS GIGANTEUS) COMME MODÈLE D'ÉTUDE
DE LA MUSCULATURE LISSE VENALE
ET DES MÉCANISMES D'AUTORÉGULATION
DE LA CIRCULATION PÉRIPHÉRIQUE

To
MY DEVOTED FATHER

whose one object in life has been the
promotion of my knowledge

To
MY LOVING MOTHER

and To
Professeur H. HUGGEL

This Book is Dedicated

I would like to express my sincere gratitude to Professor Hansjörg Huggel, Director of the Laboratory of Comparative Anatomy and Physiology, University of Geneva, who accepted me into his Department and kindly undertook to direct my thesis. His enthusiasm and un-failing moral support throughout this period were vital to the completion of this work.

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horizon of our research; his expertise and friendship have proved invaluable. Professor Owman has also honoured me by accepting to act as a member of the Jury.

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P R E F A C E

The mechanisms involved in the regulation of vascular tone and blood flow in different regions have been the subject of considerable speculation in early years and of controversy in the later ones. Though modern histochemistry and novel methods for recording electrical and mechanical activity have greatly contributed to the understanding of vasomotor and myogenic autoregulatory flow mechanisms, detailed studies of the passive and excitable properties of vascular smooth muscle as well as of the ionic events involved in these actions are as yet very scarce. Due to the difficulties which still exist in sustaining 'normal excitability' and in recording the activity of both 'perfused' (in vivo) and 'isolated' (in vitro) blood vessels in a suitable artificial medium, relatively little research has been devoted to the effects of physical, chemical and hormonal stimulation of these tissues, especially in situ. Such activities have long been known to differ considerably from one area to another, where various parameters and specialised mechanisms may combine to regulate flow through special regions such as the brain, kidney, hepatic system and erectile organs, as well as through peripheral systems involved (apart from returning blood to the heart) with heat loss, energy distribution and exudation of metabolic wastes.

The purpose of this paper is to examine the metacarpal vein of the bat wing as a new model for the study of peripheral autoregulatory flow mechanisms and venous smooth muscle in situ and in vitro. Though, in the bat, the former of these is of a rather specialised nature and, possibly, of more limited interest, the latter has been found throughout this research to possess innumerable qualities, highly advantageous to the study of vascular smooth muscle and of considerable interest to circulatory research and pharmacology.

The tendency of certain vessels to exert spontaneous rhythmical contractions, and their usefulness in studying vascular mechanisms, has been extensively described in different territories. Such 'vasomotion', as it is termed, may be found in the umbilical vessels of Man, in the anterior mesenteric veins of cats, rats, rabbits, guinea-pigs and baboons, in the sub-cutaneous arteries of the dog, in the aorta and portal veins of rats and rabbits, in the turtle vena cava, as well as in the veins, venules and pre-capillary arterioles of the bat wing (Axelsson et al, 1967; Cuthbert et al, 1965; Johansson and Ljung, 1967; Mislin, 1941; 1951; Nicoll and Webb, 1946; 1955; Roddie, 1962; Sutter, 1965 etc.).

In relation to the above, the tissue most widely studied until now has been the portal vein of the rat, and one is indebted to the pioneering studies of Johansson and his collaborators at Lund (Sweden) for most of the present knowledge in this field. However, the highly complex design of the electro-mechanical response in vitro (see e.g., Axelsson et al, 1967; Johansson et al, 1967; 1968; 1968a etc.) and the immense difficulty to be found in comparing the results with those obtained in situ with this vessel (due to its size, shape and inaccessibility) have still left many fields uncharted.

INTRODUCTION

The rhythmical and spontaneously active metacarpal veins of the bat wing are sub-divided into independent functional units (Peristiany and Huggel 1971) possessing intricate mechanisms for controlling blood flow (Peristiany and Huggel, 1972; 1973). These venous segments are endowed with several properties of singular interest to the study of peripheral venous smooth muscle and circulatory flow mechanisms.

Among these properties may be listed:

- a superficial and two-dimensional organization within a highly transilluminate and accessible skin layer,
- a well defined extrinsic and intrinsic innervation (Lane, 1972; Edvinsson, Huggel, Nielsen, Owman and Peristiany, 1973).
- a high frequency and regularity of vasomotion, also highly reproducible in vitro,
- a vessel length of several centimeters, very thin walls and the absence of a vasa vasorum (which allows the tissue to be studied in the absence of the influence of a haematological irrigation),
- a strict correlation between the excitatory and contractile processes, the presence of a 'pacemaker potential' and of action potentials of the 'plateau type' (Huggel, Johansson and Peristiany, 1973) and,

- some remarkable and apparently uniquely specialised mechanisms for aiding flow and adapting to changes in ambient conditions.

It has been stated by Speden (1970) that the relative proportion of circular to longitudinal muscle within blood vessels may not only vary from one territory to another but differ even in its physiological properties, within the same artery or vein. Since the metacarpal bat vein is entirely void of longitudinal muscle fibers (Lane, 1972) its contractile activity is limited to a helicoidally arranged muscle layer, whose physiology is still largely unexplored.

A detailed analysis of the blood and serum properties of two species of Megachiroptera (Peristiany, Lane and Huggel, 1969;1971) has led to the formation of a physiological solution C.S.3., which was tested in vivo and in vitro, and found capable of restoring normal activity and 'excitability' (with no external support) to the perfused digital veins of these animals. It has thus been possible to investigate directly (and not by topical application alone) the influence of several haematological, pharmacological and physical parameters - on smooth muscle function and autoregulatory flow mechanisms - in the total absence of any influence arising from the surrounding circulation.

Due to the wide scope of this research, the results recorded in this paper will be limited to some of the physical, hormonal and ionic factors which may influence the behaviour of this tissue, and which serve to demonstrate the effectiveness of the model chosen for such studies.

A photometric method has been developed for recording a large number of parameters including flow rate, frequency and amplitude of vasomotion as well as changes in basal tone, of perfused vessels in situ (Peristiany and Huggel, 1973). Other factors such as temperature, pO_2 , pH, ionic environment and perfusion pressure, may be controlled at all moments.

A sucrose-gap technique, as adapted for usage in vascular research (Axelsson et al, 1967), has been used to examine the correlation between electrical and mechanical activity as well as the influence of several parameters on these processes.

Fluorescent and other forms of histochemistry have served to determine the anatomical distribution of catecholamine and cholinesterase activity within a dense nervous network situated in proximity to, as well as within, the muscle layer of the venal wall (Edvinsson, Huggel, Nielsen, Owman and Peristiany, 1973). The effects of short and long term denervation at the proximal (metacarpal) and distal (brachial) levels on the activity of the 'ground plexus' have been examined in relation to some physiological effects, which result from this denervation.

Some importance has also been given to the significance of the 'stress-strain' relationship of the vessel walls to their dynamic behaviour, as well as to the influence of these parameters on pressure-flow experiments.

A brief outline will appear, after the introduction, on the nature of basal tone and the local mechanisms which control it. This short treatise will serve as a model with which to compare and classify the nature of our results.

C H A P T. I

SOME FUNDAMENTAL PHYSIOLOGICAL CONSIDERATIONS

The nature of vascular tone

The majority of blood vessels are normally maintained in a state of partial contraction known as basal tone. Vascular resistance is held in this manner at a level from which it may either increase or decrease according to the activity of the smooth muscle elements in the walls.

According to Mellander and Johansson (1968), in skeletal muscle, engaged in the maintenance of body posture, tone is due to an asynchronous or tetanic activity of individual motor units. In vascular smooth muscle, however, basal tone may be established by one or more mechanisms in different parts of the vascular tree. These processes are usually associated with an integration of 'phasic twitches', initiated by action potentials, and giving rise to contractions of a rhythmical or prolonged tetanic nature.

The resting potential of vascular smooth muscle is quite low, of the order of -30 to -65 mv, and the functional characteristics of the muscle, as well as the way in which it develops basal tone, depend to a great extent on how the action potential is triggered and, upon the possibilities for electrotonic spread of excitation to neighbouring cells.

Smooth muscles which display rhythmical activity usually continue to do so when separated from their extrinsic innervation (Bulbring et al,1963; Axelsson et al,1967; Biamino and Johansson,1970 etc.), though the role played by the autonomic nerve plexus surrounding the vessels is still not clear (Folkow,1964; Mellander and Johansson,1968). Vasomotor nerves, in the company of local control factors, probably play a secondary 'modulatory' role here, impulse generation and spread of excitation being due to innate myogenic mechanisms alone. Action potentials in such tissues may arise abruptly (Marshall,1959), or as the result of slow membrane depolarization (Biamino et al,1969; Huggel, Johansson and Peristiany,1973) similar to cardiac sinus-node pacemaker potentials.

Smooth muscle groups showing no tendency to spontaneous automatism and synchronization of contractions, do not display evidence of 'cell to cell' propagation. Tone, is here the result of asynchronous 'twitch-like' contractions, arising synchronously with action potentials triggered in different groups of cells by vasomotor nerve fibers. Denervation of such vessels, contrary to most cases in the first group, leads to loss of tone and extensive vasodilatation. One notable exception is the rhythmically-active metacarpal vein of the bat wing which, though belonging to the first group, exhibits a certain loss in tone following acute denervation (Peristiany, Lane and Huggel,1969; Peristiany and Huggel,1971).

There is also evidence, though this is rare, that parts of the vascular tree may develop tone through slow graded contractions in the absence of spike potentials. Such is claimed to be the case, for example, in the rabbit pulmonary artery (Su, Bevan and Ursillo,1964) where sympathetic nerve action has been shown to evoke tension development without triggering spikes and without even giving any measurable change in resting potential.

The possibility of developing 'tonic contracture' in the absence of corresponding membrane electrical activity, is probably extended to several vascular tissues, as demonstrated by experimental results obtained in response to changes in ionic environment either alone, or in conjunction with neurotransmitters, angiotensin, caffeine etc., (Bohr, 1964; Kjellmar, 1965; Somylo et al, 1965; etc.), The possibility, therefore, of such mechanisms participating in the maintenance of tone, under certain circumstances, should not be overlooked.

It has long been recognised that the functions of peripheral blood vessels are controlled through local as well as through remote mechanisms. The means whereby such vascular functions are controlled through adjustments of the level of 'tone' in different vessels will be discussed, while special attention will be given to the properties and behaviour of vascular smooth muscles involved in autoregulatory flow mechanisms.

The 'autoregulation of blood flow' has been called "the tendency of an organ to maintain constant blood flow, despite changes in arterial perfusion pressure" (Johnson, 1964). This definition will be extended here to include a large number of variables, apart from blood pressure (including pH, temperature, osmolarity, release of metabolic intermediaries etc.), all of which may play a role in the distribution and rate of blood flow through different vascular beds.

Lastly, it should be noted that the visco-elastic properties and tension-stress relationships of vascular walls may substantially affect the properties and behaviour of blood vessels as a whole. This factor seems to often have been neglected in the literature in relation to other physiological

behaviour, and an attempt will therefore be made to review some of the major consequences of this influence in the discussion.

Local control mechanisms

Local mechanisms of circulatory control seem more often to be concerned with the establishment of optimal exchange conditions for metabolic needs. Since this is essentially brought about by the appropriate distribution of flow through capillary beds, it is evident that both resistant vessels and precapillary sphincters are important targets for such control systems (Mellander and Johansson, 1968).

However, other vessels which exhibit high degrees of inherent myogenic automatism, such as the portal and anterior mesenteric veins, the human umbilical vessels and the bat metacarpal vein, are basically autonomous in function, and therefore highly sensitive also to local factors of regulatory control.

A great deal of conflicting evidence may be found in the literature concerning the relative importance and mechanism of action of different parameters which control such activities. The greatest controversy probably revolves around the ionic requirements for spontaneous excitation and contraction coupling, as well as for those which are brought about through external stimulation, e.g., electric current, neurotransmitters, high-K medium, histamin, angiotensin, bradykinin etc.

One possible cause of this discrepancy lies in the different experimental methods used, though another reasonable explanation might be that different mechanisms determine the

excitatory and contractile mechanisms of different vascular smooth muscles (Huggel and Peristiany,1973). The greatest difficulty that remains probably lies, therefore, in classifying these various types of response.

Bozler's early attempt in 1948 to separate smooth muscles into 'multi-unit' non-propagating types controlled by vaso-motor nerve fibers (in which group he placed all vascular smooth muscles) and 'single-unit' propagating types responsible for such myogenic phenomena as are characteristic of visceral smooth muscles, probably gave rise to more confusion than enlightenment over many years. Isolated strips removed from vessels displaying rhythmical activity remain spontaneously active when suspended in a suitable medium in vitro. This rhythmical behaviour must be due to the synchronization of contraction in different cells (Johansson and Ljung,1967; Johansson and Bohr,1966) and indicates, therefore, that separate cells in such tissues can nevertheless communicate through cell to cell propagation. It is evidently false, therefore, to classify all blood vessels in the 'multi-unit' group.

Folkow's postulate (1964), whereby a differential organization of the vascular wall may be sub-divided into 'multi-unit' and 'single-unit' layers, of which the latter alone would be responsible for non-neurogenic tone and myogenic phenomena, seems at first to be quite reasonable. The problem thus evoked, however, is whether this differentiation is histological, or merely functional. It is difficult to envisage, for example, the existence of such a 'functional' separation in vessels such as the bat metacarpal vein, where the entire muscle layer is arranged in a similar helicoidal pattern. Most recent investigation, by means of fluorescent histochemistry (Huggel, Edvinsson, Owman, Nielsen

and Peristiany, 1973), demonstrates, furthermore, that the rich adrenergic plexus of this vessel ending in varicose terminals penetrates the entire muscle layer to form a three-dimensional network terminating at the boundary of the elastic intima, and is not confined exclusively, as previously thought, to the medio-adventitial border.

Apart from these general suppositions, two separate factors recur frequently in the literature and appear to be widely accepted as determinants of local mechanisms of blood flow:

- chemical and physical factors related to cell metabolism, and
- myogenic reactions related to stretch.

Responses to mechanical stimulation, pressure and 'stretch'

The possibility that variations in blood pressure could act as direct stimuli on vascular smooth muscle, intensifying or diminishing its activity, was first postulated by Bayliss (1902) and has since formed the basis for the 'myogenic' theory of autoregulation and maintenance of basal tone.

Basically, Bayliss's hypothesis centered around the observation that, both in vivo and in vitro, increases in flow pressure gave rise to an increase in tone, whilst - given that the muscle was already in a state of tension - decreases led to relaxation and vasodilatation.

Certain objections to this theory, as an explanation of autoregulatory phenomena have been raised, on the grounds that

such 'myogenic' reactions would lead to further increases in pressure, thus stimulating the muscle to further constriction, etc. Another argument (Gaskell and Burton, 1953) has been that a 'maintained contraction' would eventually abolish its own stimulation, leading to the paradoxical situation whereby the vessel may oscillate around a diameter no different than the one existing prior to the increase in pressure. However, since neither of these two phenomena really occur, one must either conclude that this theory is nonsensical, or that certain 'brake mechanisms' are involved in modulating the response (Folkow 1964).

Ever since the disproportionately small change in renal blood flow, as a function of arterial pressure, was first clearly demonstrated (Winton, 1932), the form of nature of this autoregulation has been uncertain. Until 1965, renal autoregulation was thought to be basically due to the viscous behaviour of intrarenal erythrocytes (Kinter and Papenheimer, 1956), or to humoral 'vasoactive material' derived from an extra-renal source (Brull and Louis-Bar, 1950).

In this context, it is interesting to note, that both Mislin (1951) and Huggel (1959; 1962) considered vasomotion in the bat wing to be at least partly under the control of a 'serum factor' (arginine) circulating in the blood. It was not until 1958 that the isolated dog kidney (Waugh 1958) and later in 1969, that the isolated metacarpal bat vein (Peristiany, Lane and Huggel, 1969; 1971) were both found to function adequately in the presence of suitable artificial perfusates, in the latter case produced following the thorough analysis of the blood and serum properties of each species.

The findings of both Bozler (1948) and Bulbring (1962) in both uretral and intestinal smooth muscles respectively, that even a slight increase in longitudinal tension is associated with an increase in spontaneously generated spike potentials and tension development imply that myogenic responses are closely associated with cell membrane stability and the tendency to fire spontaneously in a repeated manner. Such responses to passive stretch are now known to be innate characteristics of all visceral smooth muscles of the 'single-unit' type. The possibility, however, that vascular smooth muscles, of a similarly 'propagating type', could also respond in such a manner, was largely neglected until quite recently. Responses of rhythmic smooth muscle from small subcutaneous arteries of the dog's paw (Johansson and Bohr, 1966), as well as similar responses recorded in this paper (p.) and obtained from the isolated metacarpal bat vein (Huggel, Johansson and Peristiany, 1973), both suggest that this reaction might be a general 'inherent' quality of some such muscles also. This observation strengthens the correct classification of 'rhythmically active' vessels into the single-unit group.

The exact manner in which 'stretch' elicits cell depolarization has not been truly elucidated. Chronic denervation and local anesthesia have been used to rule out the possible participation of nervous vasomotor reflexes in governing these reactions (Folkow, 1949; Folkow and Öberg, 1961; Hanson and Johnson, 1962; Wiedeman, 1966; Peristiany and Huggel, 1971). This does not exclude a modulatory influence that both local and central reflexes may exert on blood flow in certain regions.

It seems generally accepted in most smooth muscles that

once membrane stability is somehow affected, calcium is then the ion carrying most of the inward current during the rising phase of the action potential (Bennet 1967; Abe 1968; Brading, Bulbring and Tomita 1969; Axelsson et al, 1967). Certain notable exceptions to this rule exist, however, and will be considered in a later section concerning the ionic regulation of vascular smooth muscle function (see discussion).

In relation to the effects of stretch on vascular tissue, much less importance has been attached to the other original finding of Bayliss (1902) that a sudden reduction in transmural pressure may also elicit a sharp dilatation of the vessel. A brief reference to such an effect was again made by Nicoll and Webb (1955) following the occlusion of a wing vessel of the bat. In this case, however, the vessel was not perfused and this vasodilatation might well have resulted from tissue hypoxia or a local accumulation of acid metabolites.

A more thorough analysis of the normal venogramme of wing vessels in this animal (Peristiany and Huggel 1971, 1972) has led to the discovery that occasional reversals occurring in the normal sequence of the contraction cycle, may possibly give rise to a 'suction-like' mechanism which could aid flow under certain circumstances. This activity was later found to be related to a sudden drop in transmural pressure (Peristiany and Huggel 1972), and strongly suggests that more intricate responses to the stimulus of 'stretch' may also be involved in the peripheral regulation of blood flow.

Vascular responses related to cell metabolism

The adaptation of nutritional blood supply to the metabolic needs of a tissue is largely due to peripheral circulatory adjust-

ments brought about locally in response to changes in concentration of chemical factors related to cell metabolism. Functional hyperemia - or the tendency to increase regional blood flow during periods of increased tissue metabolism - was first postulated by Gaskell (1877) and by Roy and Brown (1879). Since then, a great many chemical substances have been adopted and, after further study, rejected as being the primary mediators of such local blood flow regulation (Berne 1964).

The fact that an increased energy-release from active cells tends to be accompanied by the liberation of acid metabolites, for a long time led to the belief that decreased pH, or the accumulation of CO_2 and other metabolic products, were the obvious agents responsible for local vasodilatation (Rushmer 1965). However, of a great variety of metabolic intermediaries examined, none seems adequate to account, per se, for the increased blood flow such as occurs in skeletal muscle during exercise, when the regional blood distribution may suddenly increase by 400-500% (Mellander and Johansson 1968).

Fleisch and Sibul (1933) investigated the vasoactive properties of a large number of organic-acid intermediaries of carbohydrate metabolism on the resting hind limbs of cats. They found these acids to elicit much greater responses when perfused in their free form (i.e., lactic acid, acetic acid) rather than when bound in conjunction with sodium salts. They, therefore, concluded that the effects observed were more likely to be due to changes in pH than to direct responses to specific anions. Kester and his team (1952) found alkaline buffers to produce vasoconstriction in skin, whereas free hydrogen ions were sometimes able to induce vasodilatation. However, changes in pH, well beyond that of the physiological range, could not

produce changes in flow comparable to those which normally follow 'exercise hyperemia' in the extremities.

To test the possibility that carbon dioxide might be a factor affecting blood flow, Guyton and his investigators (1964) perfused the hind limb of the dog with arterial- $p\text{CO}_2$ as high as five times that of normal, and found little change to result in blood flow.

Neither metabolites, pH or $p\text{CO}_2$ may therefore be considered as playing a key role in local vascular control mechanisms.

In 1964, Guyton and his associates also studied the effects of oxygen saturation on local blood flow, by perfusing either venous, arterial or a mixture of both these bloods, into the hind limbs of dogs. In this manner, they were able to demonstrate that vasodilatation occurred whenever arterial oxygen saturation was decreased. Two years later, the same author reported that a limb whose oxygen supply had been exhausted (by occluding the main artery) showed no tendency to reactive hyperemia when perfused with hypoxic blood. This could indicate that O_2 supply was intimately connected with local vasomotor control, but did not rule out the possibility that inhibition of smooth muscle could result from some 'vasodilator substance' released from the tissue by anoxia (Mellander and Johansson 1968). In vitro studies such as those carried out on aortic strips by Detar and Bohr (1968) strengthen the belief, that a lack of oxygen (or factors secondary to hypoxia), may at least be partly responsible for the establishment of functional hyperemia.

In 1941, Dawes reported that potassium induced vasodila-

tation in skeletal muscle. He reasoned that since potassium was released from active muscle, it could well be itself a mediator of local vasodilatation. This idea has received considerable support from more recent studies by Kjellmar (1961, 1965) who found potassium levels in venous blood to increase largely during exercise hyperemia, and vasodilatation to result in skeletal muscle, after infusion with similar concentrations.

The inhibitory effect of high K on vascular smooth muscle must appear paradoxical, considering the increased activity one would expect to result from a displacement of $[K^+]_i / [K^+]_o$ such as leads to depolarization. Such a 'transitory' effect has also been noted in isolated preparations of the rat portal vein, when potassium content in the bathing medium was suddenly increased (Johansson and Bohr 1966; Johansson and Jonsson 1968). However, the unprecise nature of such responses, render sceptical the exact role that K could play in local control systems.

A most recent theory specifies that changes in regional osmolarity, such as those induced by osmotically active particles released from contracting muscle fibers, could be associated with vasodilatation during exercise hyperemia (Mellander et al, 1967; Gray et al, 1968; Stainsby and Fregly 1968). Most of the weight of this argument again lies in the investigations of Johansson and his associates, who examined the permeability properties of the rat portal vein under various conditions in vitro (Johansson and Jonsson 1967; Arvill et al, 1969; Johansson 1969, 1971; Jonsson 1970). It was suggested by these authors that alterations in cell volume, besides having an influence on the concentrations of intracellular ions, might also affect the permeability characteristics of the cell membranes. A direct correlation was found in the portal vein between spontaneous activity

and cell volume: shrinking of the cells was associated with hyperpolarization and inhibition, swelling, with depolarization and increased activity. In this manner, it may be understood how an increase in regional osmolarity (due to by-products released by the cells) can lead to shrinking and thus vasodilatation. Further studies in situ revealed also that the extent of vessel dilatation recorded during experimental hyperosmolarity could well resemble that of exercise hyperemia recorded at comparable levels of venous osmolarity.

It can be concluded from this brief outlay that hypoxia, hyperosmolarity (and possibly potassium also) may contribute either alone or synergistically to enhance local responses to changes in metabolic activity. Functional hyperemia seems at present, however, to have been most closely studied in relation to skeletal muscle. Much further investigation is necessary before a more general set of rules can be established for other tissues also.

Vascular responses related to ambient temperature

The responses of vascular smooth muscle to changes in local temperature have been most closely studied in the terminal vascular beds of the bat wing. These beds offer a unique model for examining the role played by peripheral vessels in the control of heat dissipation and hence of body temperature (Hock 1951; Hanus 1959; Stones and Wiebers 1964; Henshaw 1970). Huggel (1962) found the isolated metacarpal vein of Macrochiroptera to increase its rate of vasomotion exponentially between temperatures of 4 and 40°C. This was later confirmed in vivo by Wiederhielm (1967) although no attempt was yet made to measure any variations in vessel diameter. Nicoll and Webb (1955) found the

increase in vasomotion resulting from heating the wing to be unaffected by local denervation.

Kluger and Heath were able to initiate vasodilatation in *Eptesicus fuscus* by means of internally heated rectal thermodes (1970) and heating of the Preoptic Anterior Hypothalamus or PCAH (1971). Lesions in the PCAH region, however (1971a), led to only slight thermoregulatory deficits, and the bats continued to respond to internal 'heat-stress' by peripheral vasodilatation. Such results as these, though certainly interesting, do not define unfortunately whether peripheral responses are due to nervous stimulation or to local accommodation through heating of the circulating blood. Denervation, and isolation of such vessels from their surrounding circulation in situ by newly developed techniques (Peristiany and Huggel 1973), could help to resolve such problems and to understand better the tonic and vasomotor responses of vascular smooth muscles to local changes in temperature.

C H A P T. II

SERUM ANALYSIS AND THE ELABORATION

OF A PHYSIOLOGICAL SOLUTION

Luchsinger tried as early as 1881 to maintain vasomotion in the isolated wing of the bat by perfusing it with beef's blood. Various attempts, since then, to replace the blood in an isolated bat vein by means of a physiological solution, have been handicapped by an inadequate knowledge of the serum properties of the animal's blood. Consequently it has always been impossible to sustain vasomotion for more than a few moments in the absence of an additional tissue extract (Mislin and Helfer 1958) or diluted beef's serum (Huggel 1959), mixed into the solution.

A detailed analysis of the serum ions, pH, glucose and protein contents of the blood of two species of Megachiroptera (*Rousettus aegyptiacus* and *Pteropus giganteus*), have each led to the formation of a physiological solution (C.S.1. and C.S.3, respectively) which was tested both in vivo and in vitro, and found to be capable of maintaining, over several hours, the normal excitable properties of the tissue under investigation.

It has thus been possible to examine directly the influence of several haematological, pharmacological and physical parameters on peripheral smooth muscle function and autoregulatory flow mechanisms, in the total absence of any interference arising from the surrounding circulation.

M E T H O D

A total of 16 animals in the first group (Rousettus) and 12 in the second (Pteropus) were starved 48 hours previous to the analysis in order to maintain some uniformity within each of the groups examined. It is evident that this would effect the free-glucose content of the blood serum, but it was considered more important to annul the effects of variations in the quantities of ingested food. All the animals chosen were in good health and of approximately the same weight. The ratio of male to female was approximately equal.

The animals were killed by a blow on the neck. The thoracic cavity was swiftly opened, and 5-6 ml of blood were removed from the right ventricle. A small quantity of citrate was added to the syringe previous to the heart puncture.

The following standard methods were used for the serum analysis:

A. Spectrometry

a) Proteins by Biuret

b) Inorganic phosphates by Amino-naphtol sulfonic acid (Friscke and Subbarow method)

c) Glucose by ortho-toluidine (Dubowsky method)

B. Titration

- a) Chlore by $\text{Hg}(\text{NO}_3)_2$ (Schaes and Schaes method)
- b) Calcium by E.G.T.A. precipitation with blue-hydroxy-naphtol as an indicator (Reinouts van Haga method)

C. Flame Microphotometry (E.E.L)

- a) Sodium
- b) Potassium

D. Flame Spectrometry by Atomic Absorbtion (Perkin and Elmer)

- a) Magnesium
- b) Calcium

E. Microcentrifuge

- a) Haematocrite

F. Spectrocolorimetry

- a) Haemoglobin by means of Hycel reagent

G. Osmometer

- a) Total blood osmolarity by means of cryoscopy using Fiske's osmometer

H. pH-meter

- a) pH by means of a compensatory pH-meter with micro-electrode attachment.

R E S U L T S

The results of these analysis have been tabulated in figs. 1 and 2 . The ionic values of both Rousettus and Pteropus were compared with those of a random selection of 13 Mammals, including Man. It was found that both groups of bats possessed relatively high concentrations of K, Mg and PO_4 , and low concentrations of Na, Cl and Ca, and this could explain the difficulty of previous 'Ringer solutions' to adequately maintain vasomotion in the bat wing.

Ionic Values m.eq/l.	Na ⁺	Cl ⁻	Ca ⁺⁺	K ⁺	Mg ⁺⁺	PO ₄ [≡]
<u>Pteropus giganteus</u> with complement	139	110	4.6	5.9	2.7	3.5
<u>Pteropus giganteus</u> without complement	133	106	4.5	6.7	2.8	3.9
Rousettus aegyptiacus	138	106	4.7	7.9	2.9	4.6
$\bar{X}$ of 13 mammals	149	112	5.6	6.5	1.8	2.9

Fig. 1. A comparison between the ionic values of two species of Megachiroptera and those of an average taken from a random selection of 13 Mammals. In general, these results appear low in Na, Cl and Ca, and high in Mg, PO_4 and K. In Pteropus, the K concentration appeared lower in animals fed for two months with a protein-rich complement to their diet.

The physiological solution (C.S.3.)

The normal physiological solution prepared for *Pteropus giganteus* (C.S.3.) is the following :

	<u>g/l</u>	<u>milli moles/L</u>
NaCl	5,647	96,6
KCl	0,438	5,9
CaCl ₂ .2H ₂ O	0,333	2,3
NaHCO ₃	3,448	41,0
MgCl ₂ .6H ₂ O	0,267	1,3
Na ₂ HPO ₄	0,073	0,5
Glucose	2,000	10,1

The physiological solution C.S.3. is adjusted from pH 8.00, to pH 7.4 by the addition of approximately 0.16 ml conc sulphuric acid. An adequate aeration (95% O₂, 5% CO₂) is supplied.

The osmolarity of the final solution is 298 ± 3 milli osmoles, and is very similar, therefore, to that of the whole blood.

Experimental variants of the C.S.3. solution:

1. Solutions with increased K⁺ were prepared by replacing NaCl with equimolar amounts of KCl.
2. K⁺-low and K⁺-free solutions were obtained by substituting sucrose for KCl on an equiosmolar basis.

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3. Ca⁺⁺ -low, -free, or -high solutions were prepared by adding or subtracting respectively equiosmolar amounts of sucrose.
4. Na⁺ -free solutions were obtained by substituting Tris-cl for Nacl also on an equiosmolar basis.

Effects of nutrition on the serum analysis

Animals caged for periods exceeding two months, were found to display symptoms of nutrient deficiency (frequent loss of hair, dry texture of fur, skin lesions and slow healing of wounds). A second group of bats was also examined after the addition of a nutrient adjuvant "NAFAG" (Futterzusatz für Fleischfresser No.168, Gossau, S.G.) rich in vitamins, fats and proteins to their diet. This latter group improved in health and was found, after a further period of two months, to possess significantly higher values in serum proteins, haemoglobin and haematocrite. This represented, an increase of 18%, 21% and 22% respectively (fig. 2).

The C.S.3. solution prepared from this final analysis, was tested in vivo and found to preserve intact the mechanical and excitable properties of perfused metacarpal veins over several hours (fig. 3) (Peristiany, Lane and Huggel 1969;1971).

IONIC VALUES		meq/l	Na ⁺	Cl ⁻	Ca ⁺⁺	K ⁺	Mg ⁺⁺	PO ₄ [≡]
WITH NAFAG			139 [±] 4	110 [±] 2	4.6 [±] 0.7	5.9 [±] 0.9	2.7 [±] 0.3	3.5 [±] 1.6
WITHOUT NAFAG			133 [±] 10	108 [±] 5	4.5 [±] 0.4	6.7 [±] 0.8	2.8 [±] 0.4	3.9 [±] 1.4
GLUCOSE		1.5 g / l			ACIDITY		7.38	
PROTEIN		68.8 g / l			OSMOLARITY		315 m.Osm	
PHYSIOLOGICAL SOLUTION in g/l		NaCl: 5.647 KCl: 0.438 CaCl ₂ ·2H ₂ O: 0.333 NaHCO ₃ : 3.448						
		MgCl ₂ ·6H ₂ O: 0.267 Na ₂ HPO ₄ : 0.073 Glucose: 2.00						

Fig. 2 The results of a serum analysis of two different populations of *Pteropus giganteus*, the first with, and the second without, a protein-rich complement (NAFAG) added to the diet over a period of two months.

The values of the physiological solution were deduced from the serum analysis of the protein-fed group. The osmolarity of the C.S.3. solution is 298[±]3 milli osmoles, and very similar, therefore, to that of the whole blood.

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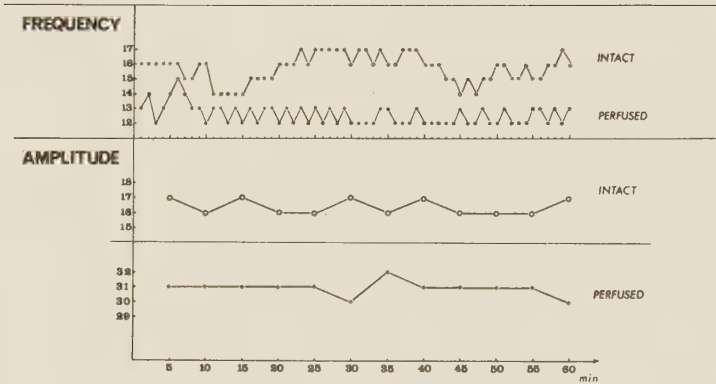


Fig. 3. A comparison between rhythmical vasomotion recorded in intact and C.S.3. -perfused digital bat veins. Greater chronotropic regularity in the perfused preparation may be due to a steadier input pressure resulting from the absence of continuity with the arterial pole. The rhythm of vasomotion is unchanged after one hour of perfusion with this medium. Previous 'Ringer solutions', recommended in the literature, did not function adequately in the absence of additional serum extracts.

C H A P T. III

METHODS FOR RECORDING DYNAMIC ACTIVITY

A. The photometric measurement of venomotion

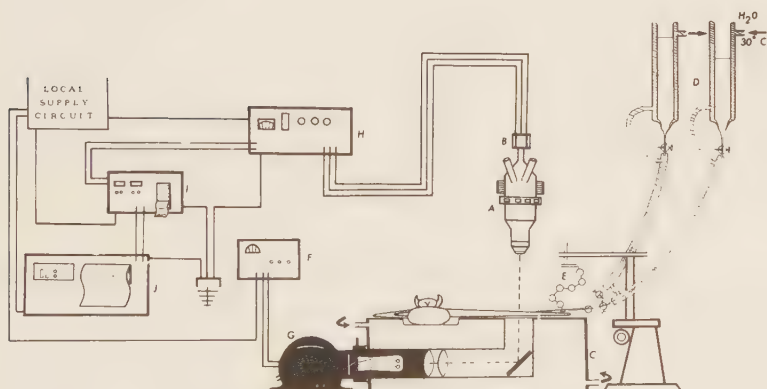


Fig. 4. Schematic diagram of photometric circuit used for recording venomotion:

- A. Stereoscopic binocular M5 Wild - B. Double photoresistor (Clairex CL730L/2) - C. Operating chamber - D. Thermostatised graduated cylinders - E. Prior micromanipulator - F. Transformer - G. Mercury lamp (projectina 5561 C) - H. D-C bridge amplifier - I. Frequency counter (Sodeco) - J. Sefram servotrace.

M E T H O D

The bat is narcotised with Nembutal (50 mg/kg I.P.) and heated 5-10 minutes under a 60 watts lamp during a brief period of pre-narcotic muscular hypersensitivity. After 1½ - 2 hours, 0,2 cc (50 mg/ml) Nembutal solution is again injected at approximately one hour intervals.

The animal is placed on its back, and a wing is spread and immobilised over an operating chamber maintained at 35°C.

A balanced and calorifically filtered mercury light beam (G) is deflected through the transilluminate wing. A section of vein, approximately 1 mm in length, is optically isolated by means of a Wild M-5 binocular incorporating a double photo-electric cell (Clairex). The current from the bridge circuit is amplified and d-c recorded in parallel onto a Sefram servo-trace ink-recorder and a frequency counter (Sodeco) modified to record the total number of contractions per minute.

Any change of diameter of the vessel acts as a diaphragm in the path of light and modifies the resistance of the photo-sensitive unit. A contraction increases (upward deflection) and a dilatation decreases (downward deflection) the total light energy reaching the cells.

Canulation and perfusion

The skin is liberated (on one side of the bone) over an area of approximately 10-15 mm in length at the distal and proximal ends of the metacarpal bone. The neurovascular bundle is covered with the C.S.3. solution to avoid drying of the tissues and the vein, artery and nerve are carefully separated from one another by means of a fine surgical thread. Special care is taken not to damage the nerve bundle lying between the two vessels.

The artery is ligated at the proximal, and the vein at both the proximal and distal regions. The vein is then canulated at both ends, leaving in between, a section 8-10 cm long. The distal canule is used to introduce, and the proximal one to collect, the perfusate (test solution).

All measurements are made at the centre of a segment between two valves. Secondary venules irrigating the anastomised section are cauterised to avoid mixing of the blood with the test solution.

The operating chamber and the 'test' solutions are maintained at approximately 30° C. The perfusates are aerated with a mixture of 97% O_2 and 3% CO_2 .

An experiment is always preceded by the recording of the activity of the intact vessel, followed by that with the 'normal' physiological solution (C.S.3.).

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B. Sucrose-gap technique for simultaneously recording electrical and mechanical activity in vitro

The sucrose-gap technique for recording simultaneously both electrical and mechanical cellular activity was first described by Stämpfli (1954) and later modified for smooth muscle by Burnstock and Straub (1958). It has since been successfully adapted to studies on various types of vascular smooth muscle (Axelsson et al, 1966; Cuthbert and Sutter 1964; Keatinge 1964).

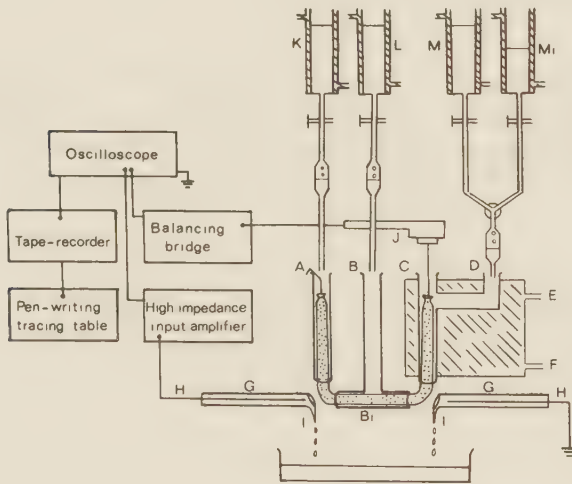


Fig. 5. Schematic drawing of a sucrose-gap apparatus and equipment used for recording electrical and mechanical activity in the metacarpal bat vein.

(After Axelsson et al, 1966). The vein is placed

in a series of close-fitting tubes A, Bi and C. The proximal end near the forearm is anchored at A and depolarized with a solution containing 128 mM K^+ (K). The distal end is connected to a force-displacement transducer, Grass FTO 3. (J) which monitors tension responses through a balancing bridge onto an oscilloscope (Tetronix 502 B). The control portion of the vein is superfused with isotonic sucrose solution (L). The recording electrodes (H), within krebs-agar filled glass tubes (G), are in contact with the 'depolarized end' and with the normally 'active end' which is superfused with 'test solutions' (M, Mi). All the solutions leave the system at the glass tips (I). The electrical readings are transmitted through a 'high impedance input amplifier' onto the oscilloscope. Both the mechanical and electrical recordings may be transmitted through a tape-recorder onto a pen-writing oscilloscript.

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M E T H O D

The metacarpal vein is sutured at both the distal and proximal ends and carefully dissected out of the narcotised bat over a length of 2 - 3 cm. All secondary vessels (there is no vasa vasorum) are cauterised to avoid collapsing of the vessel in which case the technique is not effective for measuring isometric contractions.

With some practice this procedure may be completed in 30-40 minutes. During the dissection the vein is moistened with C.S.3. solution to avoid drying. The tissue is then removed and permitted to rest in a bath of C.S.3. for approximately half an hour. During this time the Ag-electrodes are removed from the 128 mM K-solution (in which they are stored) and mounted into position on the apparatus.

The isolated vein is threaded through a series of close-fitting plastic tubes A, B₁ and C. The diameter of A and B₁ is slightly smaller, and that of C slightly greater, than that of the vessel. This process tends to anchor the vessel at points a and B₁ while allowing a free movement at C. The diameters of tubes A and B₁ are such as to permit the free passage of the relative solutions, but not the displacement of the vessel during its contraction.

The tissue is secured at the distal end (A) and 'depolarised' by continuously perfusing the tube, at a constant rate, with a C.S.3. solution whose total NaCl content is replaced by 128 mM K⁺.

The proximal end is connected by means of a fine thread to a 'calibrated' force displacement transducer (GRASS FTO₃) which

monitors tension responses in the helicoidal muscle layer. Normal C.S.3. solution, or 'test solutions', are supplied at a constant rate to this part of the vein from containers M, M₁, connected with tube D.

Water at 35°C circulates through an outer chamber surrounding C and D via E and F. All the solutions are maintained at the same temperature.

The central portion of the vein lies in the horizontal part (B₁) of the T-tube (B) which is constantly perfused with isotonic sucrose solution.

The recording electrodes consist of glass tubes filled with Krebbs-Agar gel (G) and Ag-AgCl bars (H). These are in contact with the 'active' proximal end of the vein through fluids coming from A, B, and C, and leave the system at the glass tips (I).

The outputs from the electrodes and the mechano-electric transducer are relayed through a 'high impedance input amplifier' and a 'balancing bridge' respectively, to a dual beam oscilloscope (Tetronix 502 B) and recorded through a tape-recorder onto a pen-writing tracing table.

All solutions were aerated with a mixture of 97% O₂ and 3% CO₂.

A passive, or initial 'working tension' of approximately 200 dynes, is applied to the muscle before each experiment.

C H A P T. IV

A CRITICAL EVALUATION OF THE TECHNIQUES USED IN RECORDING VASOMOTION

A. The 'in vivo' measurement of changes in vessel calibre

A great many recent studies on changes in resistance to flow brought about through the local action of drugs have been made indirectly, by determining the effects of these agents on the pressure-flow relationship of the fluid circulating through a particular organ or tissue. One important disadvantage of such methods, however, is that it is rarely possible to determine through such 'global' changes in resistance which, of all the vessels present, have responded to the drugs. Further, the erroneous assumption is frequently made that this is limited, solely to a reaction of the pre-capillary arterioles (see e.g., Wiedeman 1966).

The microscopic observation and measurement of changes in diameter of small vessels, has long been used also for qualifying the responses to drugs of different vascular territories (Grant 1930; Brun 1946; Armin and Grant 1953; Wiedeman 1954). One such technique, involving the insertion of a transparent chamber into the rabbit ear, was first tried by Sandison (1924) and later developed by Clarke et al, (1930) and by Essex (1948). Still other methods have included the observation of changes in diameter of portal venules and arterioles of rats and rabbits

by means of a fused-quartz illuminator (Knisely 1938; Hoerr 1944; Seneviratne 1949) and of isolated bat veins by means of an image projected onto a small screen (Mislin and Helfer 1958). However, though each of these methods may possess varying degrees of advantage over the other, few have been suitably combined so far with a system that is effective for distinguishing simultaneously in vivo between:

- the local responses of a single vessel-segment and those brought about through the consequences of 'haemodynamic continuity' with the surrounding circulation and,
- between changes in the parameters of flow rate, pressure and vessel calibre.

The combination of a photometric method for recording venous tone and vasomotion in the transilluminate bat wing with the perfusion of an anastomised, but otherwise intact, vessel segment in situ, has allowed us to examine simultaneously a large number of physiological attributions of venous activity in the total absence of any influence arising from the arterial pole.

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B. In vitro measurement of electrical and mechanical activity

The sucrose-gap method for recording electrical and mechanical activity was first described by Stämpfli (1954) and later modified for use with smooth muscle by Burnstock and Straub (1958). It has since been adapted successfully to studies on various types of vascular smooth muscle (Axelson et al, 1966; Cuthbert and Sutter 1964; Keatinge 1964).

Unlike the early attempts of Funaki and Bohr (1964), no direct contact exists between the smooth muscle cells and the electrodes. This by-passes previous technical difficulties and avoids the rhythmic fluctuations of membrane potential also found on occasions by these authors.

Biamino and Krukenberger (1969) have recorded the electrical activity of an electrically-stimulated rat aorta at two different platinum electrodes separated by liquid paraffin oil. These authors claim that the method is particularly useful "for the analysis of the synchronization of electrical activity, as well as for the investigation of conduction velocity and excitability", in that the potentials recorded represent the integrated activity (including the slow waves and action potentials) of large portions of the vessel strip - whereas the micro-electrode or sucrose-gap techniques reflect only the state of excitation of a small part of the preparation.

Kuriyama and Tomita (1969) have also claimed that a change in membrane potential is difficult to measure accurately by means of the sucrose-gap, since the potential change across the gap is not only due to the membrane potential, but also to the liquid-junction potentials.

Though such criticisms are undoubtedly valid, the practical advantages of the sucrose-gap technique (and its ability to record simultaneously both electrical and mechanical phenomena) seem to outweigh heavily such technical difficulties as may be found, for example, in inserting micro-electrodes through connective tissues and into a single muscle cell. Pressure electrodes, on the other hand, record electrical activity from a smaller number of cells than either the sucrose-gap method or external electrodes (Gillepsie 1962).

Each of these methods has its advantages and disadvantages, and the existing results recorded by means of a sucrose-gap method should be considered most useful when regarded as qualitative rather than quantitative because of the above difficulties and uncertainties.

C H A P T. V

THE MECHANOGRAMME

The usefulness of studying in detail the mechanogramme of any tissue that exhibits regular and rhythmical movement, is evident: Firstly, a through knowledge of the various phases and types of rhythm evoked will invariably give a deeper insight into the mode of functioning of the organ and of the dynamic retributions of the latter. Secondly, such a study will determine a basic or 'standard' activity with which later to compare experimental or anomalous behaviour.

The term "venogramme" is rather novel in itself, for it is only recently that one has escaped from the popular scientific dogma that only a passive role may be attributed to the venous system in aiding blood circulation. It has since been realised that certain vascular territories may be endowed with highly elaborate mechanisms for regulating blood flow (Nicoll 1964; Johansson and Bohr 1966; Johansson and Ljung 1967; Axelsson et al, 1967 etc.) - and one particular purpose of this paper may be said to be the demonstration of this aspect in yet another model, the pulsatile veins of the bat wing.

D'Agrosa (1970), working with Microchiroptera, separated peripheral venous vasomotion into two groups. These he appropriately termed "continuous" and "discontinuous", according to the form and duration of the relaxing phase of the cycle. This definition, however, made no distinction between the "diastolic

filling" and "resting period" components of the relaxation phase.

In order to clarify this point, and to reduce confusion between the terms "filling pressure, diastole, relaxation, resting period, return to basal-tone level" etc., it is proposed to review this classification in more detail:

Though basically similar in pattern to that of the Microchiroptera, the normal venogramme of *Pteropus giganteus* (Megachiroptera) is subject to complex variations. Some of these may be reproduced in the laboratory,

- a) following artificially simulated intramural pressure changes (Peristiany and Huggel 1972),
- b) in "fatigued preparations" perfused in vivo or suspended in vitro, and
- c) after certain changes in the ionic composition of the physiological solution.

Normal venous vasomotion is subdivided into regular (A) and irregular (B) rhythms.

The following observations have been made on approximately one hundred live 'fruit-eating' Megachiroptera (*Pteropus giganteus*) originating from Bombay, and kept in two cages of six cubic meters each at high relative humidity and at 22-24°C.

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R E S U L T S

A. Regular Rhythms

Under "regular Rhythms" are included all venogrammes in which no variation in amplitude is exhibited within either the systolic or the diastolic phases. The contraction-dilation cycle may ("discontinuous") or may not ("continuous") be separated by a "perceptible" resting period.

Continuous and discontinuous venous vasomotion may alternate periodically within the same segment with no apparent motive. The change from one pattern to another, takes place by a gradual shortening or lengthening of the iso-volumetric resting period.

a) "discontinuous" venomotion

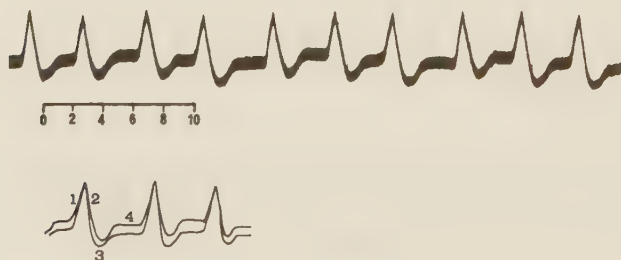


Fig. 6. Discontinuous venomotion in the intact metacarpal vein of Pteropus giganteus. The numbers 0 through to 10, represent the passage of time in seconds. The numbers 1 to 4, adjoining the schema below, represent four consecutive phases of the mechanogramme, which are the following: 1. Contraction (systole). 2. Relaxation (diastole). 3. The anisotonic resting period (which returns basal tone to its normal level). 4. The iso-volumetric (or true) resting period.

Discontinuous venomotion is characterised by the presence of a definite relaxation period which includes two separate phases: one anisotonic and one isovolumetric.

In all, four consecutive phases may be distinguished (fig. 6):

1. Contraction (systole)
 - rapid in origin, duration and passage to phase 2.
2. Dilatation (diastole)
 - surpassing basal-tone level. Phase rapid at first, then tapering off to resting period.
3. Anisotonic resting period
 - which returns dilated vessels to a basal-tone resting level of regularly oscillating amplitude (phase 4).
4. Isovolumetric resting period
 - of variable, but generally longer duration than phase 3.

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b) "continuous" venomotion

The continuous form is composed of regularly alternating contractions (systoles) and dilatations (diastoles). The duration of the systolic phase may be equal (fig. 7A), superior (fig. 7B) or inferior (fig. 7C) to that of the diastolic component. The passage from one phase to the other is smooth and without visible interruption:

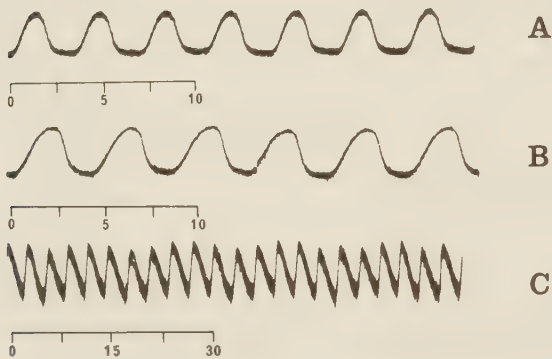


Fig. 7. Continuous venomotion of the metacarpal vein of *Pteropus giganteus*. The systolic phase is of equal (A), superior (B), or inferior (C) duration to that of the diastole. The figures 0, 5, 10, etc., represent the passage of time in seconds.

Contractions of the "continuous" type may be encountered in groups containing one to five (more rarely up to eight) cycles, each group being separated from the other by a long "compensatory" isotonic relaxation period (fig. 8). This effect is known as the "Wenkebach phenomenon" and has formerly been described in the heart of the dogfish (Wenkebach 1903).

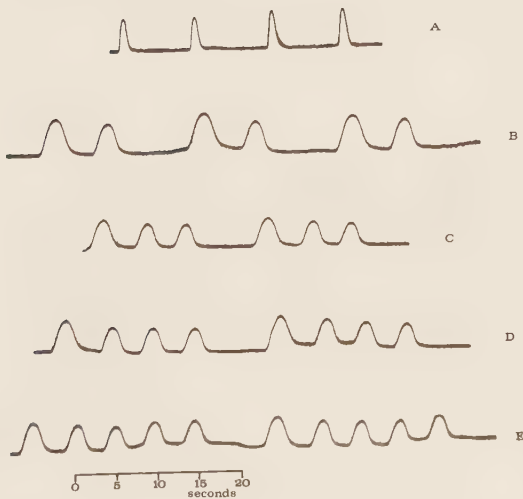


Fig. 8. 'Grouping' of contractions of the "continuous" type in closely packed numbers of one (A), two (B) etc., in a digital vein of *Pteropus giganteus*. Though occurring naturally, this effect is occasionally evident in "fatigued" perfused preparations, as well as during simulated intravascular pressure changes. A similar phenomenon has been described in the heart of the dogfish (Wenkebach 1903) as well as in the isolated frog auricle in this paper.

Similar effects have been observed in our laboratory (fig. 9) following longitudinal tension changes of the isolated frog's auricle (*Rana temporaria*).

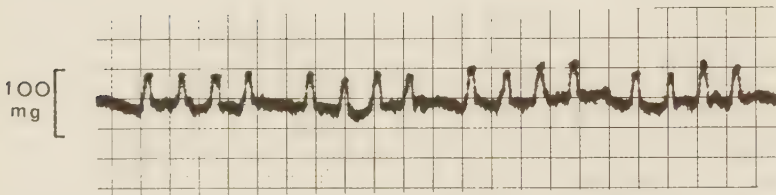


Fig. 9. Grouping of contractions in the isolated auricle of *Rana temporaria*. Each square represents two sec. This effect is known as the "Wenkebach phenomenon" and has been found to exist in several muscle-types, including the bat metacarpal vein. Though the cause is unknown, it is often observed 'in vitro' in "fatigued preparations" and may well, therefore, be related to cell metabolism.

In the digital veins of the bat, several conditions including simulated intravascular pressure changes, denervation and different changes in the ionic composition of the perfusate, have been seen to bring about this "Wenkebach phenomenon".

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B. Irregular Rhythms

Irregular activity (arhythmia) may occur as either of two different types: "transitional" or "complete". Both these forms are characterised by the interruption of a regular pattern of activity through a change in amplitude, frequency and/or basal tone.

- a. Irregular rhythms of the "transitional" type include extra-systoles and extra-diastoles. These phenomena arise spontaneously following sudden changes in systolic or diastolic pressures, and may be instrumental in restoring normal activity and flow to the vein.

N.B. It is to be noted that such phenomena have never been recorded in any other living vessel. The constant recurrence of these under normal conditions, however, and the ability to reproduce them artificially following input vascular pressure changes, confirms their presence and may point to their haemodynamic significance.

- b. "Complete" arhythmia is associated with the total disruption from any basic rhythm. For unknown reasons, a vessel is periodically unable to adapt to any definite rhythm. This may simply be due to a desynchronisation of contraction.

Extra-systoles

An extra-systole (E-S) appears in the majority of cases after a premature contraction of low amplitude (fig. 10 E-S 1). The E-S is never preceded by a resting period, and, in contrast to cardiac extra-systoles, is not followed by an "Compensatory" resting period.

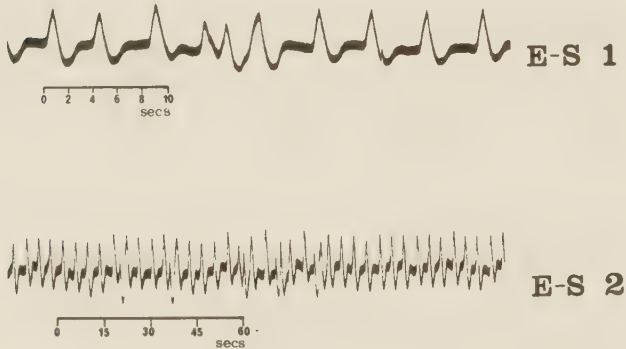


Fig.10. One (E-S 1) and several (E-S 2) Extra-systoles in the intact digital vein of Pteropus giganteus. This effect has also been reproduced following abrupt rises in perfusion pressure and is believed to be associated with local mechanisms of flow autoregulation.

The systolic contraction may be of variable height, whilst the diastolic amplitude is always superior to that of the preceding one, and tends to increase the diastolic filling volume. The following contraction re-establishes the normal systolic level of activity.

Extra-diastoles

It has also been observed, in the intact preparation, that a rapidly rising basal tone, accompanied by a diminishing diastolic filling phase, may result in the appearance of a varying number of Extra-diastoles (E-D):

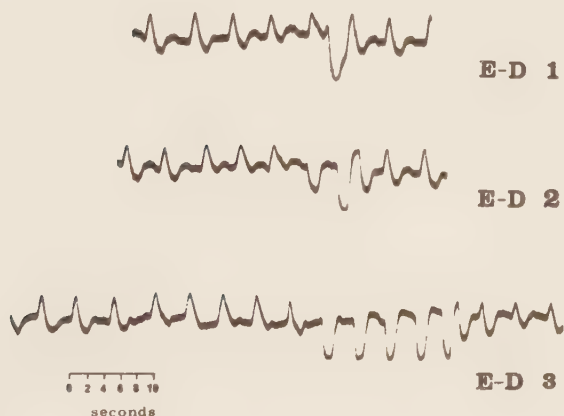


Fig.11. Extra-diastoles of the metacarpal vein of *Pteropus giganteus*. Note one (E-D 1), two (E-D 2) and five (E-D 3) extra-diastoles compensating previous insufficient filling phase.

Extra-diastoles (fig. 11) are distinguished by the complete reversal of the normal contraction-dilatation cycle of the mechanogramme (diastole - systole - isovolumetric resting period). Total diastolic volume displacement may increase by 100% with no noticeable change in frequency of contraction.

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Given that the normal phases of the mechanogramme are reversed, that the diastole replaces the systole and that the segment is closed at the proximal end by a valve, it is believed that this "diastolic contraction" may act as a 'suction-pump' and participate actively in aiding flow (Peristiany and Huggel 1971,1972).

C H A P T. VI

THE PASSIVE AND ACTIVE RESPONSES TO STRETCH OF THE ISOLATED VEIN IN VITRO

Most biological materials display, when stretched, an abrupt rise in "passive" tension, followed by a gradual decay of this tension, to a new level. This reflects the passive physical properties of the material (Sparks 1964) and is usually explained on the basis of the elastic and viscous properties of both the contractile and non-contractile components in the tissue.

Various authors have demonstrated, however, that a further "active" tension is also developed by some smooth muscles of the 'single-unit' type after an appropriate stimulus or 'stretch' has been applied (Bayliss 1902; Bulbring 1955; Hilton 1962; Gordon and Nogueira 1962; Folkow 1962; Sparks 1964; Peristiany and Huggel 1972). It has been found that this reaction is related to an interference of the cell-membrane stability leading to enhanced spike-firing and, hence, contraction. The 'active response' of such muscles may be said to represent the basic mechanism for local myogenic regulation of blood flow.

The responses of the metacarpal vein to stretch in vitro were examined with a view to defining the nature of these reactions, as well as to relating them, where possible, to various dynamic phenomena incurred in vivo - in particular in response to changes in perfusion pressure.

M E T H O D

Vessel strips, $3 \pm 0,5$ cm in length and approximately 1 mm in diameter, were suspended in vitro and superfused with C.S.3. - solution which had previously been shown to be capable of sustaining normal rhythmical activity for 4-5 hours. The solution was aerated with a mixture of 97% O_2 and 3% CO_2 and maintained at approximately $35^{\circ} C$.

The proximal end of the vessel strip was immobilised and the latter end was relaid by a fine thread to a force transducer (GRASS FTO3) whose electrical activity, representing tension of the strip, was amplified through a modified wheatstone-bridge amplifier and recorded onto a pen-writing oscillographe.

The transducer was mounted on a moveable support which permitted adjustments of the passive tension to within 0,2 mm - each step of which represented a change of 40 mg or dynes/cm². An initial tension of 200 mg was applied to the vessel. At this tension the vessel contracted regularly at a more or less similar rhythm to that occurring in vivo (12 ± 4 /min.) The vessel strip was allowed to 'rest' for one hour in C.S.3. solution before experimentation, to help overcome the trauma of mounting and dissection.

Each increment in passive tension (stretch) of 40 mg was rapidly effected and spaced at 5-minute intervals. Each step thus represented a change of approximately 20% of the initial tension value.

Stretch adjustments were pursued until the vessel strip was no longer able to either an active contracture or any spontaneous vasomotion (i.e. approx. 640 dynes/cm²).

R E S U L T S

Tension-length relation following regular increments in "quick" stretch.

Due to the passive and active components of the vessel wall, there is a dual, biphasic, mechanical response to "quick" stretch, at each increment. As will be seen, the passage from one phase to the other is time-dependent, the latent period separating which, is shorter as the passive tension rises.

Each successive increment in stretch (40 dynes/cm^2) results, initially, in an increase in length between the original and new lengths, which is here designated as the 'maximum displacement' or initial increase in length (fig. 12).

When 'vessel length' is plotted in ordinate and tension in abscisse, a typical convex curve is derived for applied forces of 200 to 600 dynes/cm^2 . It follows that the 'initial increase in length', at each increment, decreases as the tension rises. This may be attributed to the progressive increase in passive tension in the walls and is typical of any visco-elastic body that is stretched.

Following this response, certain vessels, e.g. the dog superior mesenteric artery (Sparks and Bohr 1962), then display a gradual loss or decay in tension to a 'steady state' -value higher than the tension before the strip was stretched. This is not the case for the metacarpal vein of the bat,

however, where an 'active' response follows sufficiently quickly at most passively applied tensions to mask this effect.

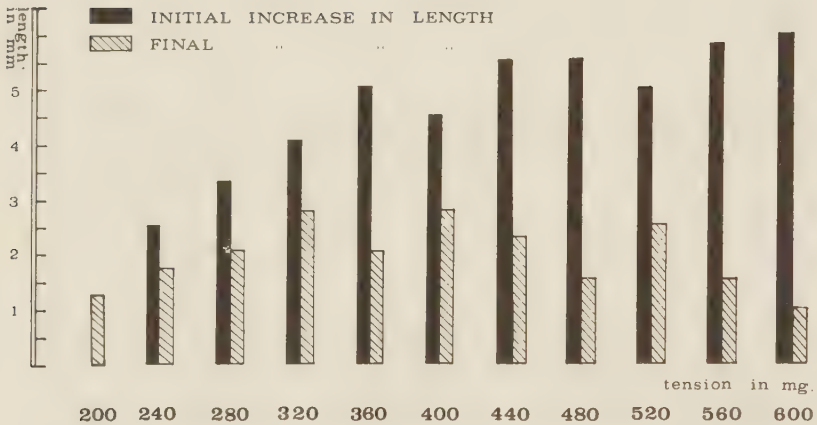


Fig. 12. The 'initial' and 'final' increases in length following the passive stretch, between 200 and 600 mg, of the isolated bat metacarpal vein. These values represent, respectively, the passive and active working - tone levels of the vessel. The first of these two is due to the passive stretching of the walls, and the second, to the development of an active myogenic contraction. Both in vivo, and in vitro, rhythmical vasomotion is seen to remain closely related to vessel length (or diameter).

Note: This apparent lack of a steady-state 'relaxing phase' following the passive stretch of the metacarpal vein may well point to a less significant role being attributed to this vessel, acting as a blood reservoir.

The 'active tension' which develops in the bat vein as a result of each increment in stretch, decreases the length of the vessel, from that of the 'initial increase in length' -level to that of a new equilibrium state, which will be called here the final increase in length (fig. 12).

If one now measures the distance between the 'final increase in length' (at any one step during the experiment) and the 'initial increase in length' -level at the following increment (which will be called: the real increase in length); and if one then plots this value against tension (dynes/cm²) on the same graph as in fig. 12) it will be seen that this value is no longer an exponential one, but becomes, on the contrary, a straight line (fig. 13).

If initial increase in length at each increment =

$$Y_1, Y_2, Y_3 \text{ etc.}$$

final increase in length = X_1, X_2, X_3 etc., and,

real increase in length = $0_1, 0_2, 0_3$ etc., then,

$$\underline{Y_2 - X_1} = 0 \text{ at any increment.}$$

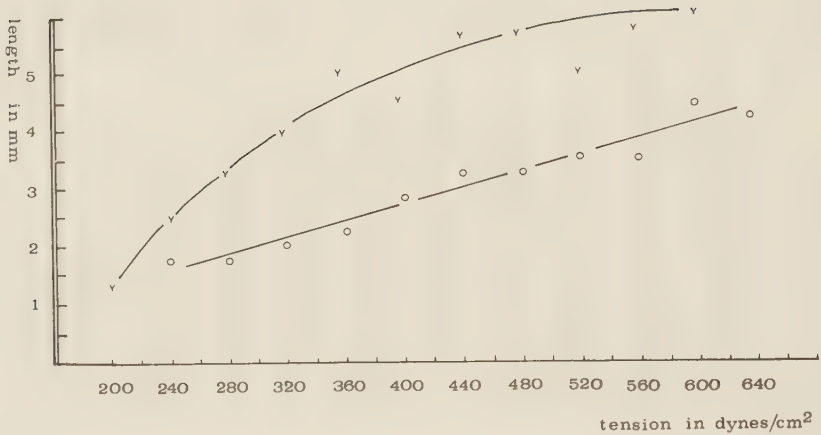


Fig.13. The 'initial', Y, and 'real', O, increases in length of the metacarpal vein, following each increment in passive stretch. The second value, which is seen to bear a linear relationship at each increment, was deduced by subtracting the final (active) tone -level after contraction, from that of the subsequent (passive) tone -level after each increment in stretch.

The final increase in length following increments in "quick" stretch

If the values derived for the 'final increase in length' (as a result of active tension) are joined at each step between tensions of 200 and 600 dynes/cm², then it is seen that this gives rise to a convex curvature, whose terminal values are very similar to those of the initial 'resting tone' -level (fig. 12).

This result strongly indicates that: no degree of passive stretch (up to 600 dynes/cm²) may result in changing the final length of the vessel to any significant extent. This is obviously of considerable advantage to the dynamic attributions of the vessel in maintaining a constant 'working tone' -level and confirms, to a certain degree, the conception of a myogenic control of basal tone in such blood vessels.

Furthermore, if a parallel is drawn here between the effects of transmural distension in vivo (p.75) and of passive stretch in vitro, it may be seen that in both cases, the rise in 'passively applied' tension is accompanied by an initial increase in chronotropy and a decrease in inotropy of vasomotion, after which both these values are reversed. It would appear, therefore, that basal tone is a primordial factor in regulating vasomotion and that vessel calibre is at all times a function and final product of both passive and active tensions.

The rate of completion and magnitude of the "active" response following quick stretch

The final increase in length of the vessel following quick stretch is a direct derivative of three different parameters:

- initial tension,
- initial increase in length, and
- the degree of 'active tension' developed.

Note: it is possible that an auxillary period of 'decay' may follow the prolonged "active response". This, however, was never observed within the five-minute interval normally allotted to each change in tension, and was not to be found either, when this period was extended on one occasion to 15 minutes.

Since no more complete analysis, other than a passing reference was found in the literature (Bozler 1947; Burnstock and Prosser 1959; Sparks and Bohr 1962; Sparks 1964) concerning the 'magnitude' and 'rate at which the active response develops', we proceeded to define this process in the metacarpal vein.

Contrary to the finding of Sparks in the human umbilical cord (1964), a "latent period" did not precede the active response in the bat vein. Active tension commenced immediately and was directly related, both in magnitude (fig. 12) and in rate of development or 'completion' (fig. 14), to the increment in passively applied tension.

Between increments of stretch of 240 and 640 dynes/cm², the increase in active tension exerted by the muscle rises, somewhat irregularly, from 300 to 2000 dynes/cm². This is accompanied by a gradual, exponential-like, decrease in the rate at which the active steady state tension (final increase in length-level) is reached.



Fig. 14. The rate of development of active tension, following passive stretch of the isolated metacarpal vein. The great rapidity of response at more elevated tensions might explain the occurrence of extra-systoles in response to 'sudden' elevated perfusion pressures.

At passive tensions surpassing 640 dynes/cm^2 , virtually no 'active' tension was evoked following stretch - this was found, by means of the sucrose-gap method, to be associated with the complete cessation of all electrical activity, and gives an insight as to the strong correlation that exists, between spike emission and active-tension development.

This sudden inability of the vein to develop an active contracture when subject to excessive stretch, might also explain the sudden drop in amplitude and increase in frequency of vasomotion exhibited in vivo, at very high perfusion pressures (fig. 15).

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The relationship of contracture tension to frequency of spike discharge and vasomotion after passive stretch

Though it is not possible to calculate the exact frequency of vasomotion during each period of 'initial increase in length' (due to the duration of the period being too short) the chronotropy appeared to increase when the vessel was stretched.

The longer duration of the 'active' response, however, has allowed us to compare the frequency of electrical discharge and vasomotion with the vessel length during each period of 'final increase in length' (see table below). It may be seen that a certain tendency exists for frequency of vasomotion to increase when the vessel length increases, and vice versa.

<u>Passive Tension</u>	<u>Final Increase in Length</u>	<u>Frequency of Vasomotion</u>
240 dyn	1,75 mm	9 per min.
280	2,00	11
320	2,75	11,5
360	2,00	10
400	2,75	12
440	2,25	10
480	1,50	8
520	2,50	14
560	1,50	12

C H A P T. VII

RESPONSES TO MECHANICAL STIMULATION

AND TRANSMURAL PRESSURE IN VIVO

A certain interest has been revived in the last few years concerning the responses of blood vessels to changes in transmural pressure. It has been thought that such behaviour as results from this procedure may lead to a better understanding of, and a greater ability to distinguish between, the following two phenomena:

- the 'autoregulation of blood flow' through certain regions by the enhanced contractile activity of smooth muscle elements in response to stretch and,
- the 'reactive hyperemia' or vasodilatation such as often follows the temporary mechanical occlusion of a vessel (Nicoll and Webb 1955; Lofving and Mellander 1956; Driscoll et al, 1964; Green et al, 1964; Uchida and Bohr 1969; Bohr, Verrier and Sobieski 1971).

A vascular bed which demonstrates autoregulation has one or more mechanisms which operate locally to maintain some variable constant (tone) in the face of variable externally and internally induced stresses (Green and Rapela 1964). Most theories of autoregulation are based on an active response of vascular smooth muscle to some aspect of this stress.

That vessels may respond to mechanical stimulation by contraction, is evident by stroking the surface of the skin. The dilatation of small vessels after mechanical stimulation of a wide area of tissue is another well known phenomenon which is usually attributed to the action of a substance (H-substance of Lewis) released from the adjoining tissue cells.

In 1921 Wachholder, and in 1944 Burgi, evoked contractions in isolated segments of carotid and mesenteric arteries respectively, by elevating the internal pressure. Since then, further support for Bayliss's hypothesis (see p. 23) has stemmed amongst others, from the investigations of Folkow (1949,1956), Waugh (1960), Green and Rapela (1964), Uchida and Bohr (1969) and of Peristiany and Huggel (1971,1972).

Fogg (1937), followed by Nicoll and Webb (1955), have reported dilatation of pial vessels of the cat and of vessels in the bat wing, upon mechanical occlusion of an artery. However, since a drop in pO_2 tension and an accumulation of certain substances related to cell metabolism have both been known to lead to similar effects (p. 23), it is difficult to attribute this result to a direct change in the contractile state of the muscle due to a decreased activity of the 'stretch' response. More recently Peristiany and Huggel (1971,1972) have examined the effects of changes in transmural pressure in situ in anastomised sections of bat metacarpal veins. They have found these vessels to respond occasionally to sudden drops in perfusion pressure by a series of strong phasic dilatations.

Though it has long been suggested that the principle stimulus for vasomotion in the bat wing is pressure within the vessel (Nicoll and Webb 1955; Wiedeman 1957; Huggel 1959; Nicoll 1964), the proper investigation of this process has long been hindered by the difficulty to maintain vasomotion in the absence of additional substrates added to the perfusate (see Chapt. II).

This investigation was carried out to determine the relation between blood pressure and flow, as well as to gain a better understanding of local myogenic responses related to the autoregulation of blood flow. An attempt has also been made to correlate certain irregularities recorded in the normal mechanogramme (see p. 55) with those which might be associated with sudden changes in perfusion pressure.

M E T H O D

Progressively increasing or decreasing 'step-changes' in flow, employing some form of constant flow system, have been described as being the most efficient means of studying the effects of intra-luminal pressure in situ (Green and Rapela 1964). Such techniques have been adopted here for use in examining the effects of transmural pressure in perfused sections (approx. 3 cm long) of metacarpal veins.

Each section was surgically isolated from the surrounding circulation to free it of adaptive changes such as might arise from areas other than those investigated.

Each increment in perfusion pressure was of the order of 5 cm water column height. All measurements of frequency and of amplitude were taken soon after the completion of the myogenic 'active contracture' which developed in response to the increase in transmural pressure.

R E S U L T S

Some general observations:

The metacarpal veins of both Rousettus aegyptiacus and Pteropus giganteus continue to display rhythmical vasomotion up to 24 hours after the surgical removal of the wing. This exemplifies the freedom of the essential process of active vasomotion from central nervous control.

A light stroking of the surface of the skin covering the metacarpal vein may elicit two to three strong phasic dilatations such as those described in Chapt. V.

If a vessel is mechanically depressed by means of a probe, a strong contracture develops beneath the probe which may persist over several minutes, bringing flow almost to a stop.

The frequency of vasomotion may differ in two contiguous segments. We attribute this phenomenon to the different number of secondary venules which irrigate the main branch at different levels, thus giving rise to variations in internal pressures.

In vitro (Huggel 1962), the isolated vein of Rousettus

may respond by active vasomotion to an internal pressure of only 2-3 cm water column height. In *Pteropus*, however, the vein remains inactive with perfusion pressures below 10 cm H₂O. This indicates that a minimum 'stretch' stimulus is necessary for activation of the contractile process.

Huggel (1959), also found pressures up to 55 cm H₂O to develop in the larger veins of the wing of *Eidolon helvum*. In *Pteropus*, a much larger species, active tension and vasomotion may still be observed at artificial pressures reaching 100 cm H₂O.

The 'tonic' responses of the metacarpal vein to 'step-wise' increments in perfusion pressure

The first response of the veins to an increase in transmural (perfusion) pressure was the development of an 'active contracture'. This may be considered as representing the myogenic response brought about through the passive stretching of the walls. This contracture was found to persist, without change, so long as the particular filling pressure was maintained (up to 15 min.) and represents, therefore, the active 'working tone' -level of the vein. The passive 'transitory tone' -level, during the initial period of entry of the solution, lasted only a matter of seconds. Chronotropic and inotropic responses of the vein were found at all times to be directly correlated to the 'working tone' diameter of the vessel.

The frequency of vasomotion increases in a more or less linear manner up to filling pressures of around 30 cm H₂O. Beyond this level, the nature of the response to variations in filling pressure may be divided into two groups, those lying within, and those beyond, what may be called 'the normal physiological range'.

- A) In vivo, this 'normal' physiological range is considered to be one in which the vein can still respond to an elevated perfusion pressure by the development of an active vasoconstriction (or increase in 'contraction tension').

If a parallel can be drawn with responses to longitudinal tension which have been recorded in vitro, this range would correspond to a passive tension application of approximately 200-600 dynes/cm².

Such an active tension was developed in vivo between the water column pressures of 30-75 cm H₂O and may be said to represent the range, therefore, at which myogenic autoregulation of blood flow is most effective.

- B) Beyond the water column pressure of 75 cm H₂O, the active vaso-constriction which developed in response to the increase in transmural pressure, rapidly declined. At 90-100 cm H₂O, the myogenic response became totally absent, and the hyper-dilated vein presumably then behaved as an entirely passive conduct.

A similar reduction in active tension development was recorded in vitro between passive tensions of 600 and 720 mg.

Chronotropic and inotropic responses to changes in perfusion pressure

Between the filling pressures of 30 and 75 cm H₂O, the frequency of vasomotion at first rises and then falls, whilst the opposite is true for the amplitude of contraction (fig.15). Beyond 85 cm H₂O, active tension is no longer developed, the vessel dilates, and the chronotropy rises accordingly.

This pattern of vasomotion corresponds exactly to that which one might expect to observe were the numerical values of these parameters solely dependent on the state of dilation of the vessel. When the vein dilates, therefore, the frequency rises and vice versa. The opposite is true for the amplitude, and in fact, a strictly exponential relation has been found to exist between these two parameters at all moments during an increase or decrease in transmural pressure (fig. 17).

Note: The behaviour, as described above, is similar in 'denerved' preparations and corresponds, furthermore, to that which has been recorded in longitudinal responses to 'stretch' in vitro.

These findings would confirm that the autoregulation of blood flow at the metacarpal level of the bat wing is entirely myogenic in origin.

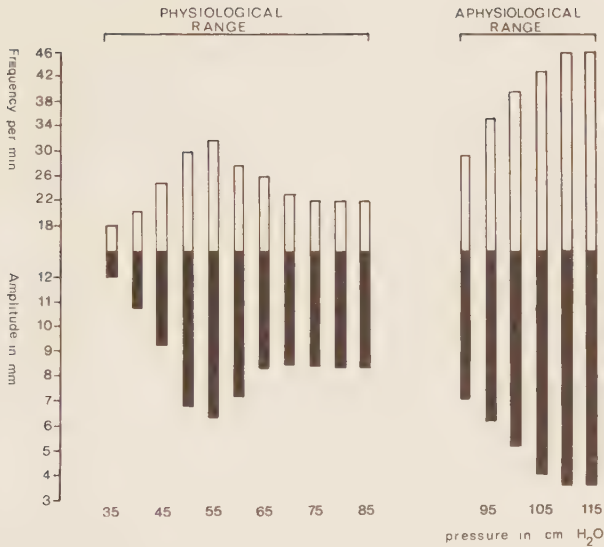


Fig. 15. The effects of step-wise increases in perfusion pressure on the parameters of frequency and amplitude of vasomotion, in the wing metacarpal vein of Pteropus, in situ. Two regions may be distinguished in the diagram: one in which an 'active contracture' develops in response to heightened transmural pressure (physiological range) and the other in which, this myogenic response rapidly declines in amplitude, leading to a pronounced passive vasodilatation (aphysiological range). At all times, chronotropy and inotropy of vasomotion seemed related to the active basal tone -level.

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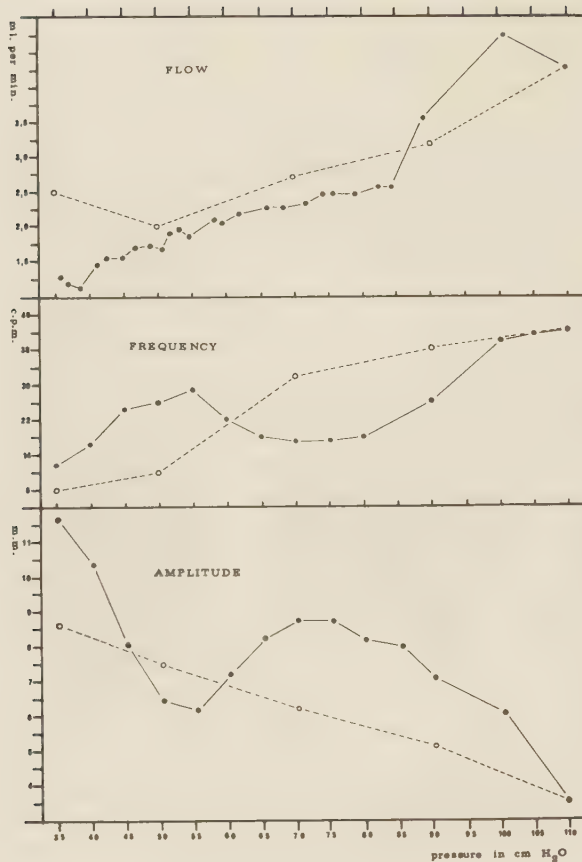


Fig. 16. The effects of increasing (continuous line), and later decreasing (dotted line), the pressure of perfusion in a metacarpal vein between pressures of 35 and 110 cm water column height. Flow is seen to bear a linear relation, and, therefore, to display autoregulation, between pressures of 35-85 cm H₂O. The effects of hysteresis on frequency and amplitude are evident in the upper pressure range where active myogenic contracture is not exerted.

The relation of 'flow' to step-wise changes in perfusion pressure

A linear relationship between flow rate and increases in perfusion pressure persists throughout what has previously been described as being the normal physiological range (fig. 16). Such a response is highly indicative of active smooth muscle adjustments leading to auto-regulation of blood flow, as, in purely passive vascular beds, the relationship between these two parameters is curvilinear and convex towards the pressure axis (Green and Rapela 1964).

Beyond the physiological range (i.e. above 85 cm H₂O), a sudden augmentation in the increase of flow may be noted (fig. 16). This is probably due to the circumference of the vessel increasing in the absence of an 'active contraction' developed in response to stretch. Whereas vasomotion appears normally to be more important than vessel diameter in determining flow rate, it is quite probable that the high input pressure which is operative at this time could impede the normal functioning of the valves. This would explain the high rate of flow recorded at the time when the efficiency of the vasomotor mechanism is impaired.

The effects of reducing the pressure in a step-wise manner after this has been raised to 100 cm H₂O

If the pressure of perfusion is first raised to 100 cm H₂O and then reduced in a step-wise manner, the

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effects of hysteresis, on the parameters of both frequency and amplitude, may clearly be seen in the pressure range above 65 cm H₂O (fig. 17).

Some evidence was obtained in vitro, that the 'active tension' developed by the vessel in the physiological range was significantly greater, after it had first been subjected to hyper-extension. In vivo, however, this parameter is difficult to measure over a period lasting 2-3 hours. Such an explanation, though, might account for the reversal of the hysteresis loops of these parameters in the lower pressure range. It is interesting, that the basal tone, frequency and amplitude of vasomotion nevertheless combine to produce a flow rate quite similar to the one arrived at during the initial raising of the pressure (fig. 16). Once again this depicts the efficiency of the autoregulatory flow mechanism.

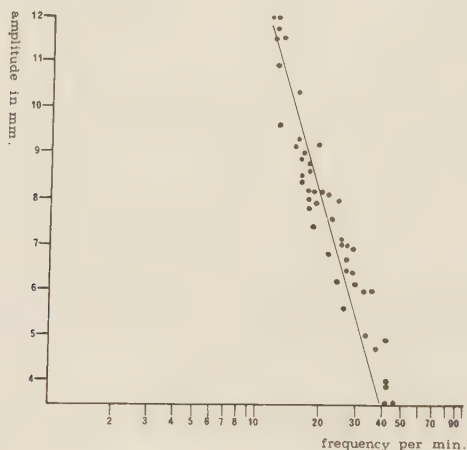


Fig. 17. The exponential relation between frequency and amplitude during simulated intraluminal pressure changes in the digital vein of Pteropus giganteus.

The effects of 'suddenly' raising or reducing the pressure of perfusion by steps of 10-25 cm H₂O

Both extra-systoles and extra-diastoles, similar in form to those observed in the intact preparation (figs. 10 and 11), were obtained immediately after suddenly raising or reducing, respectively, the pressure of perfusion by 10-25 cm H₂O (Peristiany and Huggel 1972). These responses are more probably related to the myogenic response to stretch, since a metabolic cause would presumably take much longer to take effect. Such phenomena have not been reported elsewhere in the circulatory system, other than in the case of cardiac extra-systoles. Given the nature of the response, however, they may well be a characteristic of vessels capable of rhythmical vasomotion. The functional contribution of extra-diastoles in assisting flow may be said to be twofold, firstly in filling an inadequately filled vein, and secondly in stimulating the muscular contraction of the vessel wall. This would then imply that a suction pressure or 'active dilatation' is created by the sudden reversal of the normal pattern of contraction. A relaxation or increase in diameter of arterial walls in response to a suddenly diminished arterial pressure was first reported by Bayliss as early as 1902. It was here suggested that if tone was present in response to a condition of tension, relaxation would then naturally follow if the stimulus was suddenly removed.

C H A P T. VIII

THE ACTION OF CALCIUM ON FLOW RATE, TONE, VASO-
MOTION AND NE-INDUCED CONTRACTION, IN VIVO

Ringer (1883) was the first to appreciate the importance of calcium ions in sustaining the spontaneous activity of the isolated heart. Since then it has been confirmed, in nearly all types of muscle, that ionic calcium is the essential link between the excitation of the cell surface and the shortening of the contractile proteins within the cell (Northover 1968). The extensiveness of the literature on this subject, which has been reviewed by Shanes (1958), Honig (1963), Bohr (1964), Sandow (1965) and Caldwell (1968), precludes the necessity of discussing it further within the present context.

The manner in which calcium ions are stored, released and transported from their cellular depots to the cytoplasm, has not been fully elucidated (Hurwitz and Joiner 1970) and the manner in which this occurs in vascular smooth muscle is still subject to much speculation. Hurwitz et al, (1967) and more recently, Sitrin and Bohr (1971), have suggested that calcium ions, which activate vascular smooth muscle, can be supplied from at least two sources - either directly from the extra-cellular space or from an intra-cellular depot.

The role of extra-cellular calcium in maintaining tone

and activating vasomotion will be examined in this chapter.

Some indication will also be given as to the intra- or extra-cellular source of calcium ions which are involved in the contraction brought about by Norepinephrine (and epinephrine).

R E S U L T S

The effects of variations in $[Ca^{2+}]_o$ on the rhythmicity of vasomotion in vivo

- a) Moderate decreases in extra-cellular calcium (1,00 to 0,25 mM Ca) led, almost immediately, to a reversible 'positive chronotropism' and 'negative inotropism', the amplitude of contraction becoming somewhat irregular during this time.

The complete absence of Ca in the perfusate was followed by three distinct phases (fig. 18):

- an increase in frequency and decrease in amplitude, lasting over a period of 30-40 minutes during which time the amplitude of contraction became very irregular, the standard variation of this value rising to three times that of the normal level,
- a sudden drop in both frequency and amplitude (probably due to the last remains of intraluminal Ca^{++} being washed away), and lastly,

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- the complete cessation of all spontaneous activity.

- b) A two- to three-fold increase in normal $[Ca^{2+}]_o$ content increased moderately the amplitude and decreased similarly the frequency of contraction. Higher concentration than these invariably led to precipitation within the micro-canules, and were therefore not attempted further in vivo. Biamino et al, (1968) suspended the rat aorta in a solution of 8 mM Ca, 'in vitro' (where this technical difficulty does not arise), and found that all rhythmic activity finally ceased in this medium.

The addition of $[Ca^{2+}]_o$ after a 60-minute washout of intraluminal Ca

- a) One hour after the complete cessation of spontaneous activity (following the depletion of Ca in the perfusate), half the normal content of $[Ca^{2+}]_o$ i.e. 1,15 mM, was sufficient to reinstate a normal rhythm after 40 minutes of perfusion in this medium. Since this effect was not immediate, one must assume that a certain length of time is necessary, during which the normal $[Ca^{2+}]_o/[Ca^{2+}]_i$ equilibrium across the cell membranes may return.

During this time the amplitude of contraction increased gradually, to reach about 60% of that of the initial value.

- b) On returning to normal $[Ca^{2+}]_o$, the frequency remained stable, whilst the amplitude rose by a further 20% to reach around 80% of the initial value.
- c) Three times the normal $[Ca^{++}]_o$ again increased the amplitude moderately and decreased the frequency likewise.

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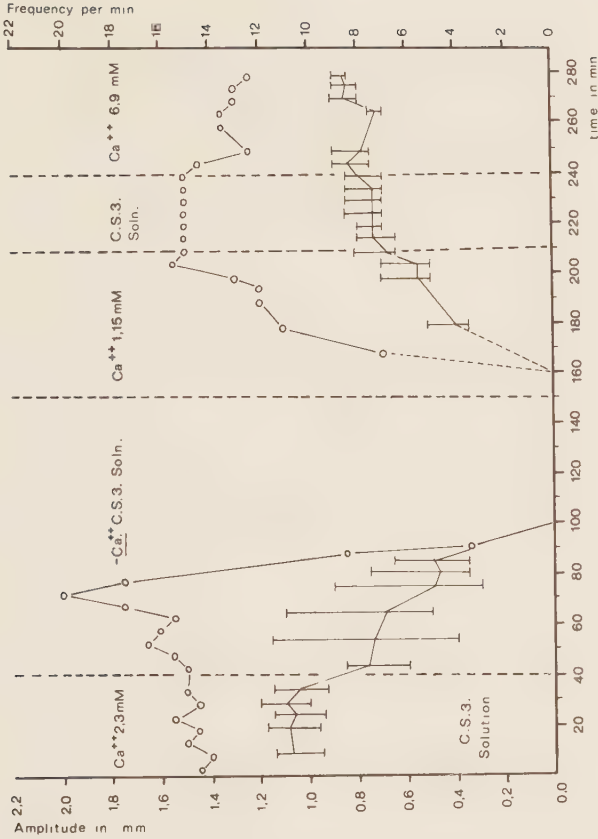


Fig. 18. The influence of total absence, half and three times the normal quantity of $[Ca^{2+}]_o$, on the frequency (discontinuous line) and amplitude (continuous line) of rhythmic vasomotion in the living bat wing (Pteropus). The vertical lines on the amplitude curve represent the standard deviation of 10 consecutive contractions. After 10 min. in Ca-free medium, flow rate was greatly diminished alongside with rhythmical vasomotion, despite acute loss of tone recorded at this time.

The effects of $[Ca^{2+}]_o$ and of Ca -depletion, on vascular tone and flow rate

A reduction of calcium in the perfusate led to vaso-dilatation and a loss in tone. This was followed in the initial stage, by a slight increase in flow rate (fig.19). Since, however, vasodilatation is accompanied at such times by an increase in the frequency of contraction, it is difficult to attribute the resulting 'increase in flow' to one or another of these two effects.

The complete removal of calcium from the medium was followed, after 20 minutes, by a short-lasting increase in flow, synchronous with the increase in frequency and loss in amplitude of contraction (fig. 19).

After 40 minutes, however, the loss in force of contraction was so great, that neither the increase in frequency or vasodilatation appeared able to impede a drop in flow rate.

60 minutes after the removal of calcium ions, both the force and frequency of contraction were greatly diminished and the flow rate dropped to 40% of the initial value, despite the considerable degree of vessel dilatation.

After the complete cessation of all vasomotion, flow rate was reduced by about 75% (to 25% of the initial value), and remained constant at that value after one hour of perfusion.

Note: These results demonstrate to what extent the rate

of flow in the metacarpal veins of the bat is not only influenced by, but very much dependent upon an active rhythmic process of vasomotion and not, that is to say, on the sole ability of the vessels to act as passive conducts.

A similar response has been observed in sequence to local denervation (see p.A-11) where a loss in tone (vasodilatation) was again accompanied by a reduction, and not an increase in flow rate. One might attribute here also; this effect to a heightened resistance of the intersegmental valves in the absence of a sufficient head-pressure developed through vasomotion.

After the vein had been washed free of calcium for one hour, the addition of 1,15 mM Ca to the perfusate increased after 20 minutes, both the amplitude and frequency of contraction to reach approximately 30% and 60% respectively of their initial values. The rate of flow increases subsequently from 25% to 60% of the original or normal value.

After one hour in this medium, though the normal frequency was re-instated, the amplitude still remained at about 70% of its usual value.

A continued increase in tone may be noted throughout this entire period and the flow rate reached 80-85% of the initial value.

On returning to normal C.S.3. solution, frequency of vasomotion returned to normal and the amplitude increased slowly to reach about 80% of the normal.

The flow rate remained unchanged, however, and it is

possible that the continued 'increase' in vasoconstriction noted during this period may have been instrumental in preventing this value from increasing.

Conclusion: the above experiments demonstrate that flow is a simultaneous function of three distinct parameters: basal tone, frequency and amplitude of vasomotion. It is also possible that the degree of 'resistance' to flow offered by the intersegmental valves may be strongly influenced by the periodical increase in head pressure brought about through active vasomotion. This conclusion is strengthened by the observation that even a strong delatation, such as follows a reduction in the calcium content of the perfusate (or denervation), does not lead to an increase in flow rate, at times when the efficiency of the vasomotor mechanism is also diminished.

Effects of Norepinephrine (NE) in normal and in Ca - deficient C.S.3. -perfusates

Both epinephrine and norepinephrine stimulate vasomotion (amplitude and frequency) and lead to vasoconstriction when applied topographically to the small arterioles and veins in the bat wing of Micro- and Megachiroptera (Nicoll and Webb 1946; 1955; Nicoll 1950; Mislin 1959; 1966; Wiedeman 1954; 1955; D'Agrosa 1970; Lane 1972). This is confirmed in this paper for concentrations between 10^{-8} and 10^{-6} M of both epinephrine and norepinephrine perfused in situ through the metacarpal vein of Pteropus giganteus. No degree of ac-

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commodation was apparent when these neurotransmitters were perfused continuously in the same concentration for a period of 10 minutes.

With a view to determining the action of NE in the absence of $[Ca^{2+}]_o$, the metacarpal vein was perfused with Ca-free C.S.3. solution until the vessel had completely relaxed (see p.90) following which an additional half hour was allowed to wash away the last remnants of intra-luminal calcium - following this, a calcium-free C.S.3. solution containing NE 10^{-6} M was perfused through the vessel:

After two minutes of perfusion with this solution vasomotion frequently returned followed rapidly by a strong tonic contracture. Over the next few minutes, the frequency of contraction increased and the amplitude decreased, as would a preparation whose calcium had been removed from the bathing medium. Gradually, vasomotion declined and the vessel came to rest in the dilated condition. The further perfusion of NE had no effect. If traces of calcium were now added to the perfusate, a strong contracture was again developed and vasomotion returned to normal (fig.20).

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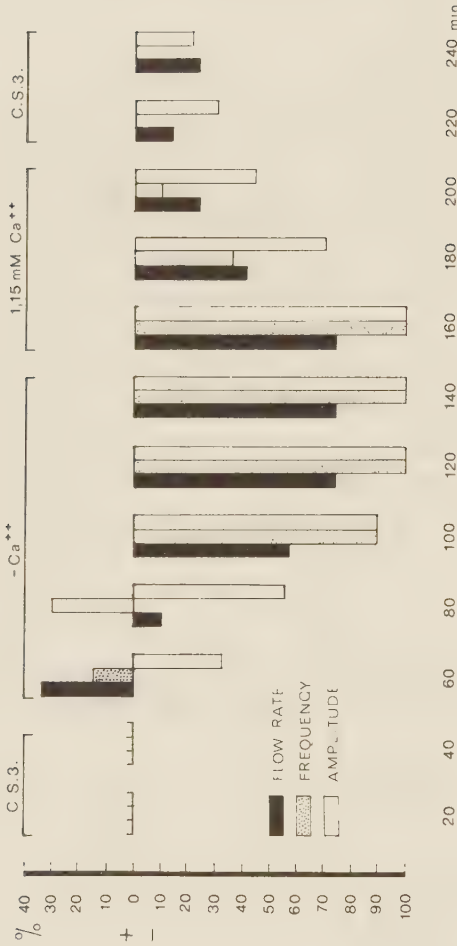


Fig. 19. The relation of flow rate (black) frequency (dotted) and amplitude (white) of vasomotion to the calcium-ion content of the perfusing solution. The initial increase in flow in Ca-free medium may be due to either, the increase in frequency or vessel calibre (not represented on graph), or to both of these. After 10 min. in Ca-free medium, flow rate diminished strongly (as did frequency and amplitude) despite loss of tone.

Effects of EGTA in normal C.S.3. at conc: 10; 5; 2; 1 mM

Effects of EGTA in Ca-free C.S.3.: followed by the addition of Ca.

Effects of EGTA in Ca-free C.S.3. containing NE 10^{-8} ; 10^{-7} ; 10^{-6} ; 10^{-5} ; 10^{-4} M

Concentrations of 10; 5; and 2 mM EGTA in normal C.S.3.-solution, immediately stopped all vasomotion and led to dilatation when perfused through the metacarpal vein in situ. Maximum relaxation of the vessel wall was reached within 1-2 minutes.

When 5 mM EGTA (or EDTA) was added to a C.S.3.perfusate containing no calcium salt, complete relaxation took place immediately. No change occurred when, up to three times the normal Ca-content, was added to the perfusate (fig. 20).

If a vessel whose activity has previously been brought to rest, by perfusion with either 5 or 2 mM EGTA, was then perfused with a C.S.3. solution containing only 1 mM EGTA vasomotion immediately returned. The relatively 'lengthy' resting periods separating each contraction initially, progressively shortened in duration, giving rise to a normal rhythm again after a small number of contractions.

Note: This indicates that the use of only 1 mM/l EGTA by certain authors (e.g., Hiraoka et al, 1968) as a means of impeding the effects of extra-cellular calcium during experimentation, may be insufficient, since this quan-

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tity is quite adequate to sustain normal vasomotion in the perfused metacarpal vein.

The action of NE when perfused at different concentrations was again tested to determine whether this agent was effective in returning vasomotion to a vessel whose extra-cellular source of Ca ions had been precipitated by means of 5 mM EGTA.

Norapinephrine proved to be completely ineffective, at all concentrations up to 10^{-4} M, in initiating vasomotion in the absence of free extra-cellular calcium, whether the tissue had, or had not, previously been washed free of intraluminal calcium.

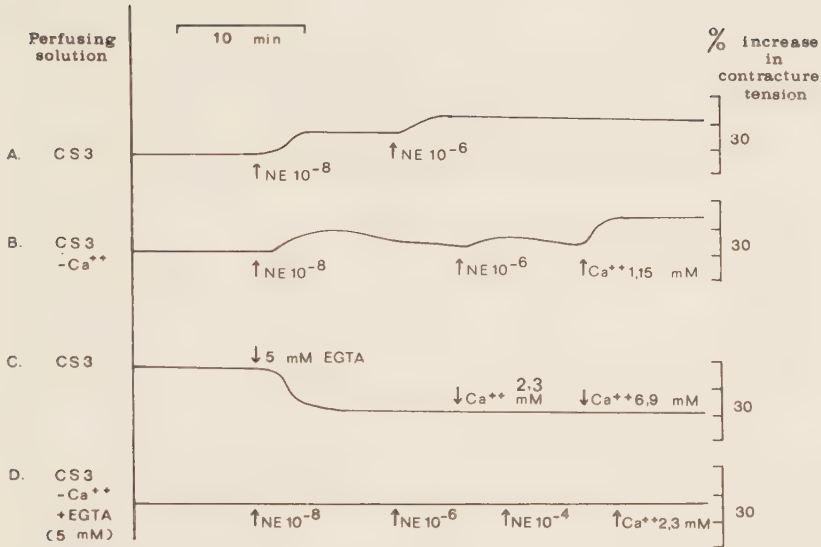


Fig.20. A diagram representing the 'in vivo' effects of the following different perfusing solutions - normal C.S.3. (A, C), Ca-free C.S.3. (B) and Ca-free C.S.3. -rich in EGTA (D) - on Norepinephrine (NE) and $[Ca^{2+}]_o$ -induced contracture tension. Note, that though NE $^{-8}$, NE $^{-6}$ may induce contracture in Ca-free solution, neither calcium or NE at high concentrations may influence tone in presence of 5 mM EGTA. This indicates that $[Ca^{2+}]_o$ is vital for the development of contracture tension in the metacarpal bat vein.

C H A P T. IX

ELECTRICAL AND MECHANICAL ACTIVITY

IN THE SMOOTH MUSCLE OF THE

ISOLATED METACARPAL VEIN

Sufficient evidence had accumulated by 1936 to indicate that the mechanical activity of most blood vessels was associated with electrical changes in the smooth muscle cells (Furchgott 1955). Luisada (1933) was able to record small fluctuations in potential with external electrodes from perfused femoral arteries of dogs. Potential changes were also observed following the sudden 'stretch' of sheep corotid arteries (Wybauw 1935).

Evidence that spontaneous rhythmic contractions of vascular smooth muscle were related to conducted action potentials was provided by Peterson (1936) who recorded transient potential changes up to 3 mv at the onset of each phasic contraction of the isolated bovine carotid artery. Each of these investigators provided suitable evidence that the potential changes observed were not artefacts arising from mechanical changes of the tissues.

Electrophysiological studies of veins have so far been almost entirely limited to those of the longitudinal smooth muscle of portal and mesenteric veins - the exceptions being Roddie's study of the turtle vena cava (1962) and Funaki's research on some amphibian venules (1958; 1960; 1961). The portal and anterior mesenteric veins of a number of mammals are 'spontaneously active' in vitro and have been extensively studied by various investigators (Cuthbert et al, 1965; Sutter 1965; Funaki and Bohr 1964; Axelsson et al, 1966; Johansson et al, 1967; 1967a). The electrical and mechanical behaviour of these veins is remarkably similar and appears to show little species variation. Spontaneous electrical activity usually arises as short bursts of 'spike-like' potentials separated by quiescent periods of more or less variable duration.

In the veins and venules of the bat wing, each phasic contraction is preceded by a single action potential arising either spontaneously (Speden and Nicoll 1972; Huggel, Johansson and Peristiany 1973) or as the result of a slow membrane depolarization resembling the activity of a cardiac-sinus pacemaker (Huggel, Johansson and Peristiany 1973).

Action potentials of the plateau-type have only been noted previously in the muscle cells of the turtle aorta (Roddie 1962). Pacemaker-type potentials have also been seen to develop spontaneously in the cells of frog abdominal arterioles (Funaki 1961) - neither of these phenomena, however, have been seen to occur previously in 'venous' smooth muscle. It is evident, therefore, that the metacarpal veins of the bat wing offer a unique opportunity for studying the excitable properties of such tissues.

The aim of the present study was to determine the kinds of electrical events which are associated with contraction in the bat metacarpal vein, and to examine briefly, the influence of some environmental factors on this process. A sucrose-gap technique previously applied to the analysis of electro-mechanical relationships in the rat portal vein (Axelsson et al, 1967) was used for this purpose.

R E S U L T S

Electrical and Mechanical Coupling in the Isolated Vein

Spontaneous mechanical and electrical activity occurred at regular intervals, at a frequency of about 15 contractions per minute, when a passive tension of approximately 200 dynes/cm^2 was applied to the vessel bathed in normal oxygenated C.S.3. solution at 35° C . Since the smooth muscle is essentially arranged in a circular manner or in the form of a close helix, circular contraction was mostly associated with an increase in the longitudinal ('isometric') force as recorded in the present experiments.

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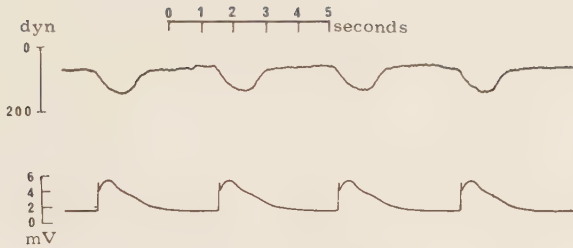


Fig. 21. Recording of electrical activity (below) and longitudinal isometric force (above) during spontaneous circular contractions of isolated metacarpal vein from a bat wing. Note the strict correlation between electro-mechanical coupling, and the precise regularity in form and frequency of both these parameters.

The normal pattern of activity observed in these veins is illustrated in fig. 21. Each contraction lasted about $1\frac{1}{2}$ seconds and was associated with electrical responses consisting of an early, rapid spike component, followed by a later slow depolarization and a prolonged repolarization.

More complex recordings were sometimes obtained from virtually normal preparations. A constant time-relationship between electrical and mechanical events remained a most constant phenomenon, however, indicating that a single pacemaker always 'drove' a whole preparation or single segment between two valves.

a) Mechanical activity:

Rhythmic contractions recorded in vitro were always of the "continuous type" such as described for normal activity in situ (p.52). Each contraction was separated or not by a 'perceptible' resting period of variable duration.

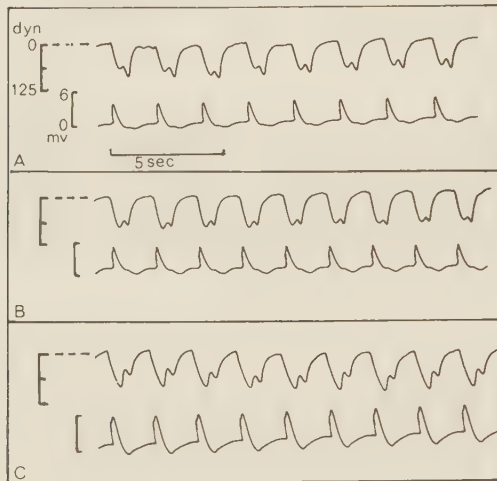


Fig. 22. 'Dual peaks', the first of which is equal (B), greater (C) or inferior (A) in amplitude to the second, sometimes recorded during the systolic phase of the mechanogramme. The first peak alone is directly related to the initial phase of depolarization.

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Dual peaks, the first of which was equal, greater or inferior in amplitude to the second, were sometimes recorded in the systolic phase of the mechanogramme. The first of these alone, was always associated with the initial phase of the spike potential (fig. 22). This observation, as well as the absence of any change in the pattern of depolarization, have inclined us to rule out the possible influence of a secondary pacemaker focus. An image such as might result from an 'electrotonic spread' from more distant portions of the vein has been observed on occasions in 'fatigued' preparations in which action potential firing was sub-threshold (fig. 23).

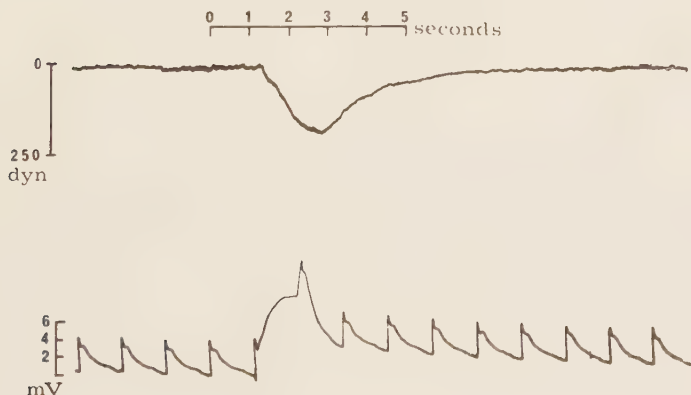


Fig. 23. Sucrose-gap recording from "fatigued" preparation of bat metacarpal vein. Focal discharge of action potentials at high frequency. A single synchronized contraction occurs in the record. This may be due to electrotonic spread from more distant portions of the vein.

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b) Electrical activity:

Action potentials arise at regular intervals, either abruptly from a steady membrane potential, or as the result of a gradual membrane depolarization, resembling the activity of a cardiac sinus 'pacemaker' (fig. 24). The former of

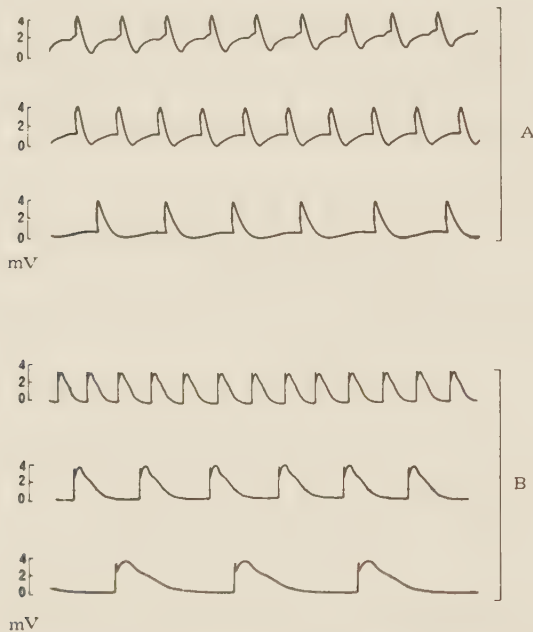


Fig.24. Two major types of electrical activity recorded in the bat metacarpal vein. In A, repetitive-firing action potentials are each followed by a slow depolarization rising to threshold level of the following action potentials. In B, action potentials arise abruptly from a steady membrane potential without any preceding slow depolarization. The latter has been called a "driven" as opposed to a "pacemaker" activity.

these types is characterised by the presence of a slow repolarization component of the "plateau type". When pacemaker potentials were present, however, repolarization was more rapid and was even occasionally followed by a 'overshoot'. Action potentials did not vary in form over long periods of time, and were each always associated with the development of synchronized phasic contractions of the muscle.

Since 'rhythmical activity' in venous smooth muscle is more often related to prolonged bursts of 'spike-like' potentials, it is suggested that the metacarpal vein might appear more suitable for the study of electrical and mechanical relationships in such tissues.

Relaxing-factor potential?

A process of 'active dilatation' has been occasionally observed in situ and has also been reproduced by suddenly reducing the inflow pressure of a perfused preparation. Sudden decreases in passive tension in vitro were also seen, periodically, to give rise to 'negative notches' in the mechanogramme (fig. 25). The electrical phenomenon which invariably accompanied this reversal of normal phasic activity consisted of a small, regular wave of depolarization. Though this apparent reversal of normal activity may simply result from a 'positive tension' development in a distant part of the preparation, it may well bear an analogy also to the phenomena observed in vivo.

In this case, it could be related to some innate mechanism of the muscle cells. In the expectation of gaining further knowledge of this process, we have temporarily named the electrical phenomenon: the 'intrinsic relaxing-factor potential'.

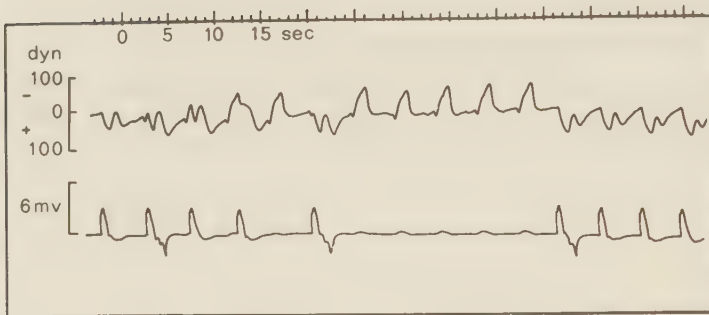


Fig. 25. Occasional 'reversal' in the normal pattern of muscle contraction synchronous with the reduction of longitudinal tension of the metacarpal vein in vitro. A small wave of membrane depolarization is seen to accompany each reversed cycle. Though these may well be artefacts, they could also be related to similar phenomena recorded in vivo following the reduction of intra-luminal pressure.

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Fatigued preparations

Various degrees of irregularities in the electrical activity, as well as of apparent electro-mechanical dissociation, were to be found in "fatigued preparations" after 3-4 hours of continuous activity in vitro. Fig. 26 shows a fairly regular activity of slow depolarizations, most of which appear to be sub-threshold. Some of these do lead, however, to firing of action potentials and to an increase of longitudinal force. In fig. 23 electrical activity is recorded from a focus with very high frequency of firing (about 40 per minute). The individual action potentials are not reflected in the mechanical recording either because there is a fused tetanus or, more probably, because the potentials fail to propagate. One strong phasic contraction occurred in the midst of this activity. The slow depolarization seen at this point could be due to electrotonic spread from distant portions of the vein.

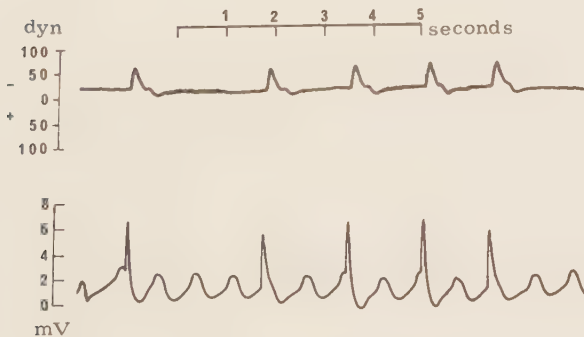


Fig.26. Recording from "fatigued" preparation.
Note the appearance of slow waves most of which are sub-threshold, and of action potentials and increases in force at irregular intervals.

C H A P T. X

SOME INFLUENCES OF CHANGES IN THE BATHING

MEDIUM ON ELECTRICAL AND MECHANICAL

ACTIVITY IN VITRO

R E S U L T S

Relationship of electrical and mechanical activity to $[K^+]_o$

a) Reduced $[K^+]_o$:

The total removal of K^+ from the superfusate led to a steady increase in the frequency of discharge, and a reduction in the duration of the quiescent period and of repolarization (fig. 28).

The mechanical events were characterized by the development of muscle contracture together with an increase in the chronotropy and a decrease in the inotropy of vasomotion.

After 5-7 minutes, 2-3 additional spike components were seen (at high speed recording) to accompany each cycle of depolarization (see fig. 27). After 10 minutes, vasomotion ceased, though multiple-peak firing continued and

could, therefore, have been the cause of the prolonged 'tetanic' contracture.

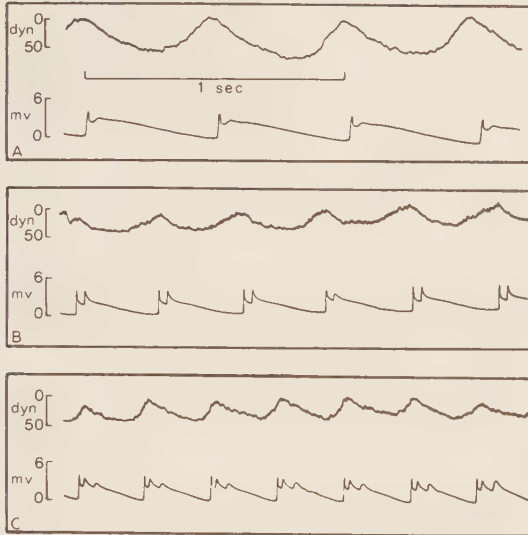


Fig. 27. Detail from the recording in fig. 28
- demonstrating how K-free medium leads to increased spike firing within the normally 'refractive' period of the cells.

Restoration of normal $[K^+]_O$, led to slight hyperpolarization, muscle relaxation and inhibition of all further discharge. After approx. 20 min., progressively longer periods of electrical and mechanical activity re-appeared. A similar loss of excitability (following a period of K-removal and return to normal solution) has been observed in heart muscle (Surawicz and Gettes 1963), taenia coli (Axelsson 1961) and in the rat portal vein.

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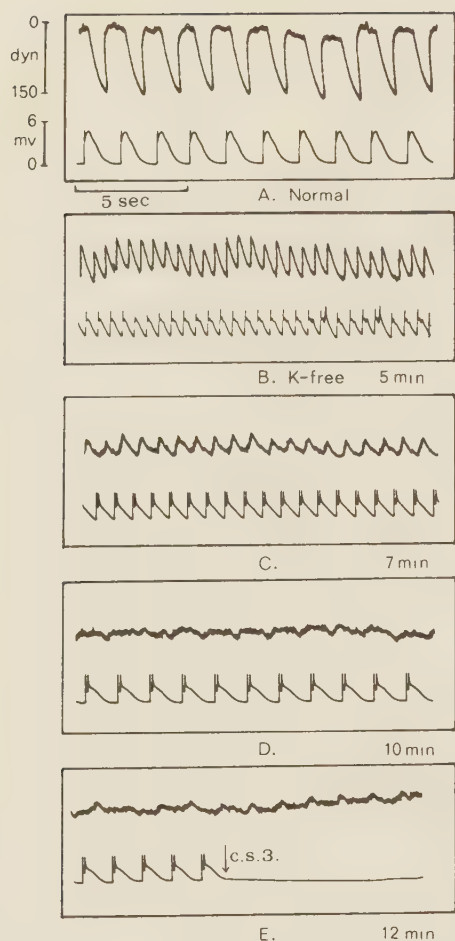


Fig. 28

Spontaneous electrical (lower tracings) and mechanical (upper tracings) activity of the metacarpal vein and its response to K^+ -free solution. A = Normal C.S.3. solution. B = Fifth minute in K^+ -free solution. C = After 7 min. D = After 10 min. E = Return to normal C.S.3. solution (arrow). A more detailed recording of the transition from single to multi-spiked potentials (between the 5th and 7th minute) is to be found in the previous figure. Note the cessation of electrical activity and the muscle relaxation which follows return to C.S.3. solution.

b) Increase in $[K^+]_o$

- A twofold increase in extracellular potassium to 12 mM K^+ led immediately to depolarization and slight increase in frequency of rhythmical vasomotion.

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- A fourfold increase (24 mM K^+) raised the membrane potential still further and gave rise to repetitive high-speed firing (fig. 29). A progressive decrease in degree of muscle relaxation was accompanied by a higher frequency of vasomotion.

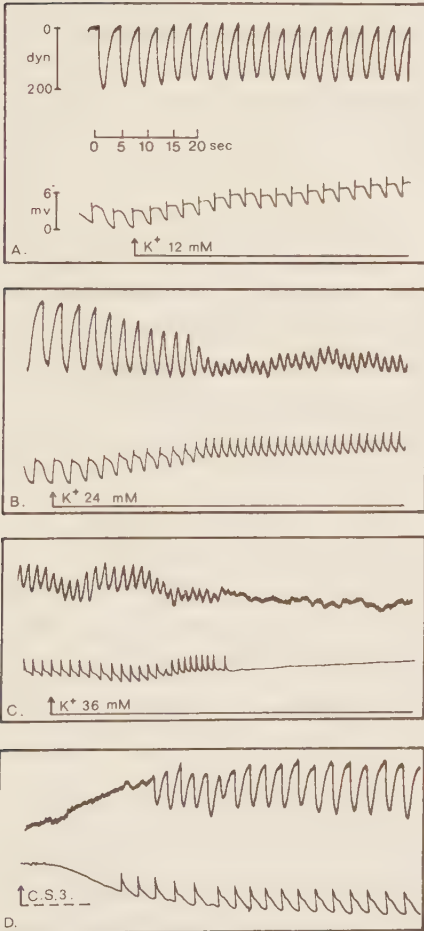


Fig. 29.

Responses of the metacarpal vein to increased extracellular K^+ concentrations. A = Normal tracing, followed by shift (arrow) to solution containing 12 mM K^+ . B = Change (arrow) to 24 mM K^+ . C = Further change (arrow) to 36 mM K^+ . D = Return (arrow) to normal C.S.3. solution. Note progressive depolarization, shortening of the relaxing-phase of the mechanogramme and the ultimate development of a maintained contracture.

- A sixfold increase (36 mM K^+) led to further depolarization and the development of a 'tetanic' contrac-

ture in the absence of any further spike activity.

- A return to normal C.S.3. solution led rapidly to repolarization and to the return of more or less normal activity.

More or less similar responses to the above mentioned, have been recorded in the rat portal vein (see e.g., Axelsson et al, 1967).

Relationship of electrical and mechanical activity to, and dependence of NE-evoked contraction on, $[Ca^{2+}]_o$

The reduction of Ca content in the superfusate to half that of normal (1,15 mM Ca^{++}) led to a distinct relaxation of the muscle and a loss in the amplitude of vasomotion.

When transferred to a solution containing no calcium (to which 5 mM EGTA was added to precipitate remaining traces of extracellular calcium), the muscle relaxed and all electrical and mechanical spontaneous activity ceased within 30 seconds (fig. 30). The muscle continued to relax gradually over the next few minutes.

At this time, the addition of NE 10^{-6} did not have an effect either on the membrane potential or on contracture tension (fig. 30). This result would indicate that extracellular calcium is essential for NE-evoked muscle contraction in this tissue.

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The return to normal C.S.3. solution was accompanied by repolarization, and a return to more or less normal activity within approximately 1 minute.

In normal C.S.3. solution, the addition of Norepinephrine 10^{-7} depolarized the membrane, increased the frequency and reduced the amplitude of both spike discharge and vasomotion. The membrane potential became increasingly unstable as these concentrations were augmented in the superfusate (fig. 32).

The simultaneous removal of both calcium and potassium from the medium led to a gradual increase in spike discharge and in rhythm of vasomotion. This was accompanied by a decay in tension and a shortening of the inotropic response (fig. 31). After 6 minutes, firing became unstable and vasomotion was greatly reduced. All activity was abolished after 8 minutes.

Returning to normal solution produced hyperpolarization and complete quiescence for 8-10 minutes. After this time, normal activity slowly returned. Such a response has also been noted in the early phase of recovery following the removal of potassium from a normal C.S.3. solution.

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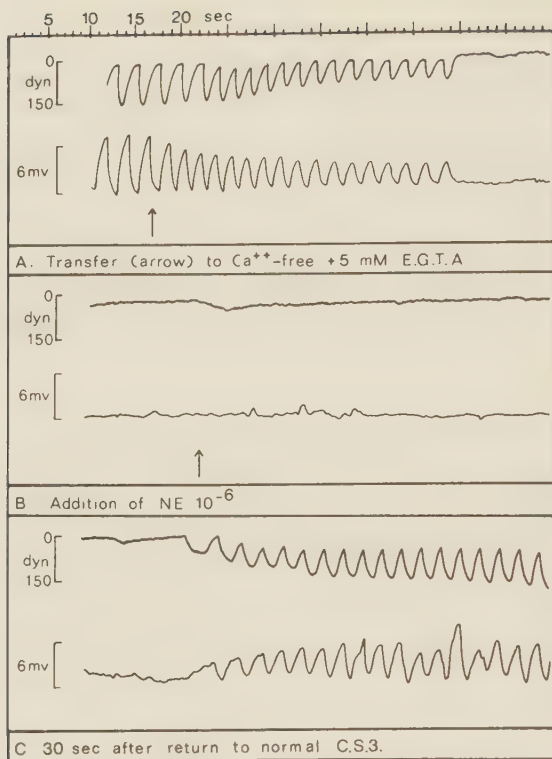


Fig. 30.

The effect of Norepinephrine (NE) in Ca-free + EGTA medium. A = normal recording, followed (arrow) by shift to Ca-free + 5 mM EGTA solution. B = addition (arrow) of NE 10⁻⁶. C = 30 sec. after return to normal C.S.3. solution. Contrary to the findings of Hirakawa et al, (1968) and of Uchida and Bohr (1969), the presence of extra-cellular calcium is essential for NE-induced contraction. These previous findings may be related to an insufficient quantity of EGTA (0,8 and 1,00 mM, respectively) being allowed for extra-cellular calcium precipitation.



Fig. 31. The simultaneous removal of calcium and potassium ions from the superfusing solution of an isolated metacarpal vein. A = normal activity. B = 2 min. after shift to K-free and Ca-free solution. C = 6 min. later. D = 8 min. later. E = 10 min. after return to normal C.S.3. Note increasing membrane instability leading to cessation of all spontaneous activity.

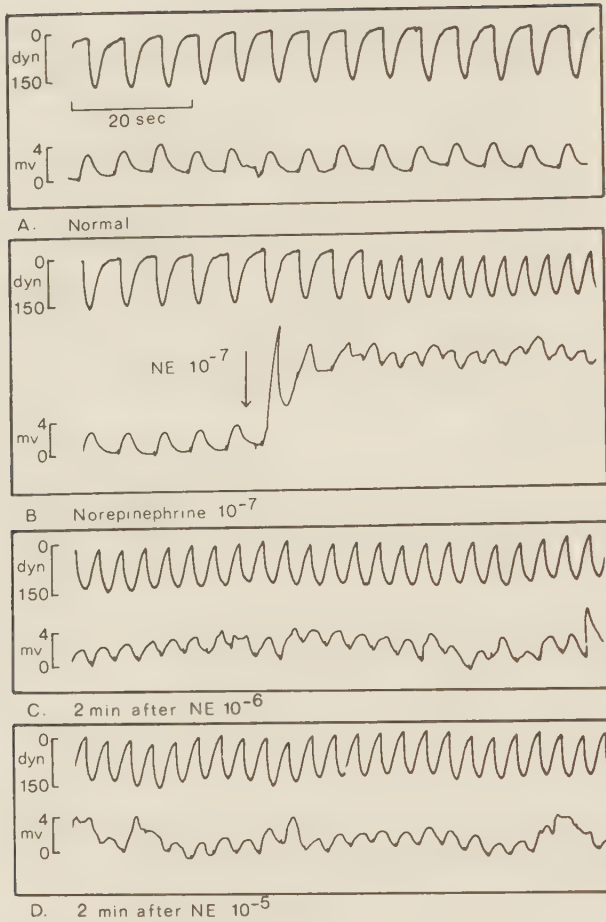


Fig. 32.

The action of different concentrations of Norepinephrine (NE) membrane potential and rhythmical vasomotion. A = recording in normal C.S.3. solution. B = addition (arrow) of NE 10^{-7} to the superfusate. C = 2 min. after shift to NE 10^{-6} . D = 2 min. after shift to NE 10^{-5} . Note increase in rhythm, depolarization and increasing membrane instability.

Relationship of electrical and mechanical activity
to the presence of Na^+ in the medium

The metacarpal vein may continue to function normally (though usually at faster rhythm) over long periods of time after the total removal of Na from the medium (Huggel and Peristiany 1973). Even more remarkable, however, appears to be the recuperative effect which the absence of this ion may exert on a muscle whose activity has been greatly reduced due to fatigue. Here, the replacement of all Na in the medium with Tris (on an equiosmolar basis) frequently led to the return of more normal rhythm and excitation-contraction coupling. In figure 34, one may follow the activity of the preparation in which all traces of Na have been replaced by Tris.

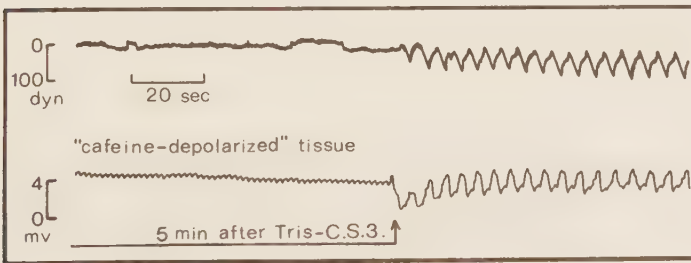


Fig.33. The effects of extra-cellular Na removal on a "caffeine-depolarized" isolated metacarpal vein.

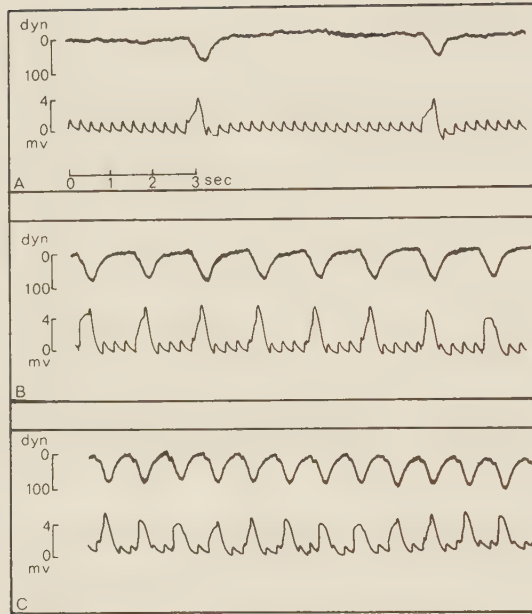


Fig. 34. The effects of Na removal (and replacement with Tris) on an isolated "fatigued" metacarpal vein. A = fatigued preparation demonstrating high frequency sub-threshold firing. B = 5 min. after replacement of Na by Tris. C = 10 min. after Na removal.

DISCUSSION AND
CONCLUSIONS

The main purpose of this paper has not been to resolve the numerous enigmas of vascular smooth muscle research, so much as to examine the utility of the model offered for such research, and the ability to relate it to studies in vivo on autoregulatory flow mechanisms.

It is strikingly evident in the literature how few parameters of sufficiently constant nature exist, on which to base dynamic vascular research. This is due to several deficiencies of both a technical and physiological nature. In a treatise on vascular tone (1961), Prof. Sharpey-Schafer, of the St. Thomas Medical School, London, once wrote:

"In the past, the main difficulty has been to find methods for the study of vascular tone ... in any such investigation there are clear advantages in finding procedures which will dissociate the behaviour of arteries and veins".

This inability to distinguish between arterial and venous responses, as well as the difficulties involved in optically isolating and mechanically recording the dynamic activity of single blood vessel, have frequently led to the misunderstanding of certain vascular functions and, especially, to the assertion, that only a secondary, passive role may be attributed to veins in aiding and controlling blood flow.

It will become increasingly evident throughout this paper that the metacarpal veins of the bat wing possess intricate myogenic mechanisms for responding to changes in local conditions, and that a considerable role may, in fact, be played by these vessels not only in aiding the return of blood to the heart, but also in 'reactive hyperemia' of the tendency to relate flow to the physiological needs of a tissue.

As we have seen in Chapt. I, various methods have been considered in the past for recording mechanical changes in vessel tone ranging from the inclusion of transparent chambers into the rabbit ear (Clarke 1931; Essex 1948), to the projection of an image of small vascular territories onto a screen (Mislin and Helfer 1958). Though the relative benefits of each one of these methods may be debated, few as yet have been combined effectively with a surgical procedure in vivo so as to allow a single vessel segment to be examined independently of the surrounding conditions in situ.

The elaboration of a physiological solution (C.S.3.) capable of replacing the blood by perfusion and the use of a photometric method for measuring transient changes in calibre of perfused, anastomised, vessel segments have played a critical role in the gathering of experimental data concerning the behaviour of these veins and their role in aiding and controlling blood flow.

The inability of early investigators to replace the blood in the wing vessels of bats by suitable perfusates (Luschinger 1881; Mislin et al, 1958; Huggel 1959) was probably due to the concentration of serum ions in this animal being particularly high in K, Mg and PO_4 , and low in Na, Cl and Ca.

A close concern for the ionic make-up and serum properties of the blood of the species used, has permitted the preparation of a physiological solution capable by itself of maintaining normal excitability and vasomotion in the wing vessels over several hours, both in vivo and in vitro (Peristiany, Lane and Huggel 1969; 1971). A further considerable advantage of this procedure has been that not only pharmaca and other agents might be added to the solution in known quantities, but also that changes in the ionic make-up of the perfusate could be effected while still maintaining the osmolar integrity of the solution. The affirmation by Nicoll (1964), therefore, that:

"humoral agents brought to the area might affect the response of muscular elements along the arteriolar vessels (of the bat wing)" but that ... "it is practically impossible to demonstrate such action", may, now, have to be reviewed.

Of the various techniques used in recording electrical responses of vascular smooth muscles, the sucrose-gap method may well be considered as the most practical and useful of all, when attempting to obtain a more qualitative information. Though this technique limits the recorded response to that arising from only a small part of the preparation, its ability to relate electrical to mechanical phenomena (over many hours) greatly outweighs such technical difficulties as may be encountered in attempting to insert and to preserve, micro-electrodes within single 'contracting' muscle cells.

The sucrose-gap method used in this study has allowed us to bring to light some remarkable inherent qualities of

the metacarpal bat vein which might be considered, henceforth, as being particularly well suited for studying the excitability of venous smooth muscle.

Two types of spontaneous electrical activity have been reported in active smooth muscles (Bülbring et al, 1958; Funaki 1961; Marshal 1963; Bennett 1966; Steedman 1966; Kuriyama et al, 1967; Speden 1973). In the first of these, 'repetitive-firing' action potentials are each followed (as in pacemaker regions of the heart) by a slow depolarization rising to the threshold level of the following action potential. In the second type, each action potential arises abruptly from a steady membrane potential without any preceding slow depolarization. This latter form has been called a "driven" as opposed to a "pacemaker" activity (Bennett 1972).

The majority of blood vessels, including the rat portal vein, periodically evoke 'spike-like' potentials of an irregular form and duration, grouped together as 'short-bursts' accompanying each muscle contraction (see e.g., Axelsson et al, 1967).

In the metacarpal bat vein each phasic contraction, occurring at highly regular intervals, is associated with a single action potential in which the 'spike component', 'slow depolarization wave' and 'repolarization' phases are each clearly distinguishable and thus separately accessible to study.

Since action potentials have been found to arise in the metacarpal vein either spontaneously or as the result of 'pacemaker-type' depolarization (Huggel, Johansson and

Peristiany 1973), it is difficult to determine, as yet, whether each single muscle cell in this vessel has the potentially to act as a 'pacemaker center' (at different times), or whether such activity is, in fact, limited to a 'specialized' region of synchronically firing cells, from which excitation then spreads, presumably, to other parts of the tissue.

Though action potentials of the plateau-type have been recorded previously in muscle cells of the turtle aorta (Roddie 1962), neither these or pacemaker-type potentials have ever before been observed in other venous smooth muscles. The bat vein may be of singular interest, therefore, in studying these phenomena in such tissues. Furthermore, the direct correlation that exists between each 'action potential' and its accompanying 'phasic contraction' enables a closer examination of the excitation-contraction coupling process to be carried out, than has hitherto been possible with the majority of other vascular models.

Various agents and ions have been found to modify the excitability and tension responses of the metacarpal bat vein:

In accordance with several other treatises on visceral and vascular smooth muscle physiology (see e.g. Bülbring et al, 1970), potassium ions seem to play a crucial role, here, in determining membrane potential and contracture tension. Changes in $[K^+]_o$ were associated with tension responses containing both a phasic and tonic component. Though it is not possible, by means of the sucrose-gap method, to establish a quantitative relation between membrane potential and the external potassium concentration, it would appear that, as

in the rat portal vein (Axelsson et al, 1967) the membrane potential was for the most part affected by the $[K^+]_o$ in a manner that might be expected, were the membrane potential at least partly determined by the gradient $[K^+]_i / [K^+]_o$.

When the $[K^+]_o$ was raised from that of normal (6mM), first to 12 mM and then to 24 mM, depolarization followed and spike firing became almost continuous. At concentrations above 36 mM, all spike activity ceased, and the phasic contractions gave way to a prolonged tonic contracture of a 'tetanic' nature. This contracture was maintained in the absence of action potential firing, and was most probably due to the increased membrane depolarization. Responses, more or less similar to these, have been recorded by others with the rat portal vein. On returning to normal bathing solutions, both these tissues recuperate adequately. The fact, however, that this process is more rapidly terminated in the bat vein, may be related to the presence of thinner walls and, therefore, of easier 'exchange' conditions obtaining in this vessel as opposed to that of the rat portal vein.

The total removal of K from the medium at first led to a short duration increase of spike-firing and of frequency of vasomotion. According to Axelsson et al, (1967), it is not yet known whether this somewhat 'characteristic' response can be explained, as has been done for cardiac muscle (Carmeliet 1960), by the presence of a reduced K^+ -conductance in the low $[K^+]_o$. During this period, the increased rate of discharge was clearly seen (when recorded at rapid speed) to be closely associated with a heightened

excitability, two to three additional spike components arising within the same action potential cycle. When Ca and K were both removed from the medium simultaneously, however, spike activity ceased as well as vasomotion.

On returning to normal C.S.3., after 15-20 minutes in K^+ -free medium, all electrical discharge was abolished and relaxation of the muscle followed (see fig.). A similar effect has been recorded in taenia coli (Axelsson 1961), in heart muscle (Surawicz and Gettes 1963) and in rat portal veins (Axelsson et al, 1967). It has been suggested that this 'inhibition' might be due to a sudden increase in the K^+ -permeability of the pacemaker-region cell membrane when the $[K^+]_o$ is returned to normal (Surawicz et al, 1963).

In conjunction with the controversy surrounding the need for adequate $[Na^+]_o$ for smooth muscle excitation and contraction coupling, it would appear that the predominance of Na-based action potentials in vessels such as the sheep carotid artery - whose cells become electrically active in simple Ca-free saline but remain inexcitable when Na in such solution is replaced by Tris or Choline (Graham and Keatinge 1970) - contrasts strongly with the behaviour recorded more habitually in both intestinal (Kuriyama and Tomita 1969) and in different vascular smooth muscles (Axelsson et al, 1967; Biamino et al, 1970; Huggel and Peristiany 1973, etc.), and is more likely, therefore, to be the exception rather than the general rule. However, further conflicting evidence whereby increases in $[Ca^{2+}]_o$ may either depress (Feinberg et al, 1962) or augment (Scott et al, 1961; Lüttgau 1967, etc.) the contractile responses and where $[Ca^{2+}]_o$ is either essential (Bozler 1968; Huggel and Peristiany 1973) or

not necessary at all (Hiraoka et al, 1968; Uchida and Bohr 1969) for norepinephrine, high-K or Mg-ATP evoked contractions, lead one to imagine that different mechanisms may be involved in determining both excitation and contraction in different smooth muscles.

As concerns the mechanism whereby norepinephrine may exert muscle contraction, various authors have reported the ability of this hormone to re-activate smooth muscle preparations in which total spontaneous activity was arrested following long periods of Ca-washout. Though similar findings to these were recorded in the metacarpal vein in vivo and in vitro, both the electrical and mechanical components of this reaction were entirely absent in the presence of EGTA or EDTA at concentrations above 2mM/l. In C.S.3. solutions containing only 1 mM EGTA, however, spontaneous electrical and mechanical activity was not impaired. This would suggest, that the use of less than 2 mM concentrations of EGTA by certain authors (Isojima et al, 1963; Hiraoka et al, 1968; Uchida and Bohr 1969), as a means of precipitating extra-cellular Ca, might be insufficient in quantity. In this case, NE-evoked contractions, as previously reported, could still have been due to the 'activation', or penetration within the cells, of remaining 'traces' of extra-cellular calcium.

According to Sitrin and Bohr (1971), the contrasting effects of altered Na concentrations reported in various preparations may be due to the differential effects of this ion on different pathways available for Ca-contraction. These may also reflect, furthermore, individualities among different smooth muscles in their ability not only to store Ca effectively, but also to release 'intracellular-bound' calcium in

different Na (and Ca) environments.

It has been observed in both perfused and isolated preparations that the metacarpal vein can function adequately, over long periods of time, in the total absence of Na in the bathing solution. Neither spike emission or contraction amplitude are in any way impaired during this time, and in fact, even more remarkably, a distinctly re-cuperative effect on both these parameters can be obtained on transposing a 'fatigued' preparation into a C.S.3. solution whose total Na-content has been exchanged for equiosmolar amounts of Tris. Such an example was recorded in fig. 34. A similar 'reactivating' effect was also obtained on one occasion, when superfusing a 'cafeine-depolarized' vein with Na-free, Tris-C.S.3. solution (fig. 33).

Biamino and Johansson (1970) have also found both spike activity and phasic contractions of a fairly high frequency to persist in the rat portal vein for 3-4 hours in Na-free Tris-Cl solution, following which, however, contraction amplitude then gradually decreased and some increase in basal-line tension was recorded. Since the rate of rise of contracture tension was found to be markedly reduced only in entirely Na-free medium - whereas the rate of relaxation was significantly affected within a wide range of $[Na^+]_o$ - these authors postulated that Na might be especially important in assisting the outward (as opposed to the inward) transport of Ca^{2+} , which occurs against the electrochemical gradient.

Following the observation by Paton and Rothschild (1965) that the calcium content of smooth muscle decreases, if the calcium concentration of the bathing fluid is reduced;

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and by Hurwitz et al, (1967) that a reduction in the extra-cellular calcium ion concentration results in an increased migration of calcium from 'cellular stores'; Sitrin and Bohr (1971) showed Ca-efflux to be depressed within a Na-free solution, and postulated that Ca released from storage-depots might remain within the cells and activate contraction.

These experiments indicate some of the complexities involved in studying the role of electrolytes in different smooth muscles as well as the difficulties that exist at this time in drawing any precise conclusions.

Despite some evidence to the contrary (Bülbring and Kuriyama 1963; Graham and Keatinge 1970), it seems generally agreed nowadays that in the majority of smooth muscles, calcium is probably the ion carrying most of the inward current during the rising phase of the action potential (Kuriyama and Tomita 1969; Biamino and Johansson 1970 etc.). According to Bülbring and Tomita (1970), furthermore, the main source providing this Ca may well be Ca that is bound at the membrane itself - where it is also intimately involved in controlling permeability to other ions. In the introductory chapter to "Smooth Muscle" (1970), Bülbring points out, furthermore, that the exceedingly small amounts of Ca involved in Ca-exchanges and the continuous transition of this ion from both a bound to a free state and from one compartment to another, all make the precise contribution of calcium in such functions particularly difficult to define.

In the bat metacarpal vein, $[Ca^{2+}]_o$ is essential for the emission of spike potentials and for both the phasic and tonic components of the muscle contraction.

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At low $[Ca^{2+}]_o$ spike discharge and frequency of vasomotion at first gradually increase and then, after longer exposure to this medium, fall rapidly leading to relaxation and cessation of all spontaneous activity. Such a 'dual' response has also been reported by Axelsson (1961) in the guinea-pig taenia coli. In the bat, the earlier of these two phases was found (in vivo) to be accompanied in part by an increase in flow rate, and this may be attributed here to either, or both, the increase in chronotropy and/or the increase in vessel calibre.

At times, however, when vasomotion is greatly reduced (either after long exposure to Ca-free medium, or soon after acute denervation) 'flow rate' is again seen to be considerably reduced. It would appear, therefore, that vasomotion is more important in determining blood flow in such 'rhythmically active' vessels than their simple ability to act as 'passive conducts'. As has already been mentioned, it is also probable (as would appear through macroscopic observation) that the 'intersegmental' valves remain closed - thus hindering flow - in the absence of the stimulus of a sufficient systolic (venal) head pressure. This is difficult to demonstrate, however, at the present time.

The difficulty of sustaining vasomotion in the bat wing by means of artificial perfusates has limited the methods used in the past for the investigation of this process to those which involved the occlusion of blood vessels - and this with the aim of enhancing or diminishing flow to certain areas (Nicol and Webb 1946; 1955; Wiedeman 1957; Wiederhielm 1967). In addition to this method, Wiedeman (1966) attempted to inject saline solutions into wing vessels and to observe the effects of changes in head pressure at more distant points, before the solutions reached the area examined. The method de-

scribed in this paper has enabled us to carry out a more precise investigation of the relationship between pressure and flow in well determined areas of the wing membrane.

Passive vascular beds have been described as showing little, or no, tendency to maintain blood flow constant in the face of a stress arising from an altered filling pressure and a minimal, or no, attempt at maintaining any other variable constant as a result of altered metabolism (Green and Rapela 1964). The relationship between pressure and flow in such 'passive' territories is usually curvilinear and convex towards the pressure axis (Whittaker and Winton 1933; Pappenheimer and Maes 1942; Green et al, 1963; 1964). According to Green and Rapela (1964), however, a linear, proportional relationship between pressure and flow is indicative of a widely dilated vascular bed or of the occurrence of autoregulation.

In the metacarpal vein of the bat the above relationship was, in fact, found to remain entirely linear within what has been termed the 'physiological pressure' or, more exactly, the range within which an active contracture is developed by the muscle in response to an increased filling pressure.

Beyond the water column pressures of 75 cm H₂O, the active contracture developed was minimal at first, and then totally absent. At this time the vein dilated and presumably behaved as an entirely passive conduct. When the transmural pressure was first raised to 100 cm H₂O, and then reduced again to 30 cm H₂O, the effects of hysteresis on both rhythm of vasomotion and flow were evident in the upper pres-

sure range only. In the lower physiological range, flow rate remained largely unchanged. These results are indicative of a considerable degree of autoregulation of blood flow in the metacarpal veins of the bat wing.

The physiological and hydrodynamic attributions of blood flow are considerably complicated, firstly, by the visco-elastic properties of the vessel walls (where passive, elastic, elements may lie both in series and in parallel to muscle cells) and, secondly, by the variations in contractile force exerted in response to both basal tone (diameter) and stretch (stress). Consequently, it has often been thought rather unethical to regard either 'wall tension' or 'vessel calibre' as the primary factor regulating (Speden 1973), or regulated by (Folkow 1964; Mellander and Johansson 1968), the myogenic response.

Whereas each successive increment in 'stretch' of the isolated bat vein has been seen to affect both the active and passive responses of the tissue, both the rate and the extent to which either of these components respond depend, still further, on:

- i. the rapidity with which the stimulus is applied and,
- ii. the initial or 'basal tone' -level that exists prior to each increment in stretch.

As a result of this, when a vein is highly distended or when the stimulus is rapidly applied, the 'active response' (contracture) may develop so quickly, that the initial or 'passive phase' is lost completely. This could explain, incidentally, how a sudden rise in input pressure can lead to a series of extra-systoles in which the passive phase or dilatation (which is normally the first response to stretch) is in no way evident.

Whatever the nature of the respective target and effector systems for the myogenic response, the influence of transmural pressure has remained largely unchallenged as being intimately related to the problem of blood flow auto-regulation (Mellander and Johansson 1968; Speden 1973). Even so, Nicoll (1964) appeared to doubt that the "sharp response" elicited in the bat wing as a result of accretion in perfusion pressure could be due to an increased volume within the segment, "since" - in his words - "equivalent filling at very slow rate will not excite a contraction".

It should be borne in mind, however, that the visco-elastic properties of the vessel walls are markedly 'time' (Attinger 1969) and 'frequency' -dependent (Hardung 1962) as manifested by stress relaxation (the decrease in stress associated with constant strain), by creep (the change in strain associated with constant stress), and lastly by hysteresis, when the vessel is alternately filled and then emptied.

Accordingly, different responses may be elicited in the rhythmically active metacarpal vein, depending on the rate and the force with which each transmural stimulus is applied. The following examples may demonstrate this fact:

1. The sudden, intensive, distension of the vein, obtained by the occlusion of close lying segments (Wiederhielm 1967; Peristiany and Huggel 1971) or by an increment in perfusion pressure (Peristiany and Huggel 1972; 1973) may result in a contraction of such magnitude, that the vessel is momentarily closed.
2. A sudden, but less intense, increase in pressure, however,

may lead to the occurrence of extra-systoles similar to those which occur in several heart preparations.

3. Finally, when the pressure change is applied gradually, the rhythm of vasomotion 'accommodates' progressively to each new 'working tone' -level, and flow is modified accordingly.

A biophysical explanation of the 'time-dependency' of certain vascular responses has been put forward by Harvey and Sparks (1964). These authors suggested that, if elastic elements lying in series with smooth muscle cells were more viscous than the latter, then "a fast pull would stretch the cells more than a slow pull", and, "the slow pull would not capture as much of the 'time-dependent' portion of the series elasticity and, therefore, would not stretch the cells as much as a rapid pull".

Another recent suggestion by Speden (1973) whereby the cell membrane itself might comprise a length-sensor element in parallel with the contractile elements, merits reflection. Such a system would readily explain how a negative feed-back is established after muscle contraction is developed and yet still remain in agreement with the findings that the myogenic responsiveness of vessels to changes in transmural pressure is most often parallel to their level of basal tone (see e.g. Mellander and Johansson 1968).

The development of active contracture has also been seen, in the bat vein, to be closely related to the basal tone -level prior to each stretch. However, flow rate in this rhythmically active tissue must be considered a function

of two separate products: vasomotion and vessel calibre. At times of severe vasodilatation (e.g. after local denervation or perfusion with calcium-free medium) the resulting drop in flow rate would seem to be a more natural consequence of the reduction in rhythmical vasomotion, than of the increase in vessel diameter. It is for this reason that it was postulated earlier in this discussion, that the intersegmental valves may remain closed in the absence of sufficient head pressure. This is consistent with the finding that a similar increase in vessel diameter, following perfusion with 'extreme' pressures above 80 cm H₂O (when the necessary pressure to keep the valves open is present), leads in this case, to a considerable increase in flow, despite the loss of efficiency of the vasomotor mechanism. The functional contribution of the valves in bat alar veins may be simply, therefore, to increase the efficiency with which intramural forces act as an auxillary pump, to propel the blood towards the heart. Muscular activity would, in this case, be transformed into 'flow energy', by the production of an immediate resistance to reverse flow after each muscular contraction.

The difficulty of presenting, at the present moment, a complete working model for the autoregulation of blood flow, is relevant to nearly all other vascular territories. Any such endeavour must take into consideration not only the functional characteristics of the smooth muscle elements and their relation to other close-lying structures, but also the superimposition of other nervous (reflex and central), hormonal and metabolic influences, which are present at all times. Nevertheless, it is hoped that the findings and the techniques reported in this paper may be considered as an incipient contribution to the problem as well as a starting point for fur-

ther investigation. The metacarpal vein lends itself as a particularly well suited model for the study of these parameters.

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R e s u m é F r a n ç a i s

Le rôle que jouent les facteurs locaux, chimiques et physiques, dans la détermination du tonus vasculaire et l'écoulement sanguin dans différents territoires a longtemps été sujet à de controverses. La raison principale réside dans la difficulté de maintenir une excitabilité normale des vaisseaux sanguins, au moyen de solutions artificielles, soit in situ, soit in vitro, et d'enregistrer leurs activités électrique et dynamique lors de l'expérimentation.

L'absence de modèles permettant l'étude des corrélations précises entre les activités électriques et mécaniques spontanées a été une des causes principales du manque de connaissance sur l'excitabilité vasculaire. Par exemple, pour la veine porte du rat (un des modèles le plus étudié à nos jours, ex: Axelsson et al 1967; Johansson et al 1967; 1967 a; 1968 ; Biamino et al, 1970, etc.) chaque cycle de contraction s'accompagne d'une dépolarisation cellulaire liée à un nombre non-déterminé de "spikes" de forme et de durée très variables. Par contre, pour les veines alaires de la Chauve-Souris, nos études de 'sucrose-gap' ont révélé que le couplage 'excitation-contraction' s'accompagne d'une seule onde de dépolarisation de forme et de durée très régulières. De plus, les diverses phases de cette activité comprennent de nom-

breuses étapes (activité "pacemaker", spike phase de plateau et repolarisation), rapellant fortement l'activité cardiaque et se prêtant favorablement à l'expérimentation. Actuellement, la veine métacarpienne de la Chauve-Souris est le seul modèle de veine connu présentant une telle activité "pacemaker" et une dépolarisation du type 'plateau' (Huggel, Johansson et Peristiany 1973).

Les veines alaires pulsatiles de la Chauve-Souris représentent un modèle idéal pour l'étude de la musculature lisse vasculaire et des mécanismes d'autorégulation de la circulation périphérique. Leur situation superficielle facilite les études dynamiques in situ. D'autre part, le haut degré d'activité spontanée de la veine perfusée in vivo et in vitro permet de maintenir celle-ci en parfait condition pendant plusieurs heures au moyen d'une solution physiologique établie, selon l'analyse hématologique.

Ce modèle pourrait certainement être utile pour d'autre recherches angiologiques. Nous avons perfectionné les méthodes de perfusion et d'enregistrement de l'activité dynamique in situ, et défini les paramètres d'activité normale. Ces activités sont très diverses et démontrent un très haut pouvoir d'adaptation locale en réponse aux variations des conditions ambiantes. Parmi les caractéristiques essentiels du mécanogramme se trouvent les rythmes normaux, 'continus' ou 'discontinus', le regroupement périodique d'un nombre variable de contractions (Périodes de Wenkebach, 1903), et la présence d'extra-systoles et d'extra-diastoles.

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L'étude des effets de la pression transmurale in vivo a nettement démontré l'importance du diamètre vasculaire, et du tonus de base, sur les paramètres de fréquence et d'amplitude de contractions. Entre 30 et 80 cm de pression de colonne d'eau, l'élévation de la pression intraveineuse par étapes de 5 cm, n'augmente que faiblement le débit et cela toujours de manière linéaire. La forme droite de la relation pression-débit par opposition à la forme curvi-linéaire est, selon Green et Rapela (1964) révélatrice d'une autorégulation locale. Nous expliquons ce résultat par le maintien d'un tonus de base peu variable en fonction de la pression de perfusion.

In vitro, nous avons vu que l'étirement ("stretch") de la veine provoque tout d'abord une augmentation de la tension passive, ce qui est dûe aux propriétés visco-élastiques innées du tissu, et par la suite une contraction tonique myogène qui ramène la longueur finale du muscle à un niveau très proche de l'origine. Une telle activité myogène in vivo expliquerait le maintien d'un tonus de base plus ou moins constant en réponse à des pressions différentes. Nous appelons "champ physiologique", les variations de tension (in vitro) et de pression (in vivo) exercées sur la veine provoquant une telle réaction biphasique. Par contre, à des tensions trop élevées, ("champ aphysiologique") la partie active myogène de cette réaction, et donc le 'contrôle du tonus de base' ne se manifestent plus. In vivo, de telles pressions élevées s'accompagnent d'une forte et soudaine vasodilatation et d'une augmentation exagérée du débit.

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Les mécanismes grâce auxquels les extra-systoles et les extra-diastoles semblent capables de compenser respectivement des pressions systoliques et diastoliques insuffisantes nous paraissent particulièrement importants. Les extra-systoles obtenues par une augmentation brusque de la pression de perfusion sont semblables à celles observées chez l'animal intact lors d'une systole prématurée et de faible amplitude. Ainsi, une stimulation plus intense, en cours de période réfractaire relative, permet à la veine d'adapter rapidement son travail aux variations de la pression intravasculaire. Par contre, si l'on provoque un affaissement de la paroi, on observe alors des extra-diastoles ou 'contractions diastoliques' pendant lesquelles les phases du mécanogramme se trouvent totalement inversées, ce qui fait travailler la veine comme une "pompe à suction". Cette dernière aurait alors une action régulatrice sur le flux veineux à la suite d'un débit insuffisant. Ceci démontre clairement l'autonomie complète de chaque segment intervalvulaire et sa faculté de fonctionner en tant qu'unité fonctionnelle totalement indépendante. Lors des changements de la tension longitudinale in vitro (avec la technique du sucrose-gap), ces contractions du type inversé apparaissent fréquemment et sont toujours accompagnées d'une onde de dépolarisation de faible amplitude de forme arrondie. On pourrait émettre l'hypothèse de l'existence d'un "intrinsic relaxing-factor potential" propre à la musculature lisse de ce tissu. De tels phénomènes n'ont jamais été décrits auparavant pour les vaisseaux sanguins. Il reste à déterminer l'importance de ces extra-diastoles dans la régulation du flux sanguin.

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Les facteurs ioniques jouent un rôle prépondérant dans l'excitabilité vasculaire et contribuent de manière significative aux mécanismes d'autorégulation locale de la circulation sanguine.

Nous avons démontré in vivo, ainsi qu'in vitro, que le Calcium a une importance essentielle non seulement auprès des composants phasique et tonique de la contraction, mais aussi pour l'émission du potentiel d'action. De plus, nous avons vu que des concentrations d'E.G.T.A ou d'E.D.T.A. inférieures à 2 mM ne changeaient ni l'activité spontanée normale, ni l'action chronotrope positive et vasoconstrictrice de l'adrénaline et de la noradrénaline. Antérieurement à ces résultats, certains auteurs ont pensé que des concentrations inférieures à 2 mM de ces chélateurs calciques étaient suffisantes pour inhiber l'action du $[Ca^{2+}]_o$ dans la contraction provoquée par la noradrénaline, ce qui a répandu une idée erronée de ces mécanismes (c.f. Isojima et al, 1963; Hiraota et al, 1968; Uchida et Bohr, 1969).

L'importance du Potassium dans le maintien du potentiel de membrane de la musculature vasculaire n'est pas contesté. Des changements en concentration extra-cellulaire de cet ion sont toujours suivis d'une excitabilité plus élevée ou diminuée, selon qu'on augmente ou qu'on réduit le $[K^+]_o$. Il est fort probable, d'après nos résultats, que l'augmentation du $[K^+]_o$, non seulement dépolarise la membrane, mais encore provoque une stimulation plus intense du "focus pacemaker".

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En général l'intervention du Sodium dans l'excitabilité de tels tissus vasculaires reste à déterminer. Quoique les travaux de Graham et Keatinge en 1970 suggèrent que la dépolarisation cellulaire soit liée à la concentration extra-cellulaire en Na, nos résultats démontrent que, non seulement cet ion ne joue aucun rôle dans le couplage 'excitation-contraction', mais encore qu'un effet net de 'récupération' est obtenu lors de l'absence sodique chez des tissus "fatigués" après 3-4 heures de travail sous des conditions expérimentales. Nous pensons donc, que pour la veine alaire de Chauve-Souris, la dépolarisation dépend plutôt d'un canal d'entrée calcique que sodique.

En conclusion, nous estimons que la veine métacarpienne alaire de la Chauve-Souris se révèle favorable aux études de l'activité musculaire vasculaire et des mécanismes d'autorégulation locale de l'écoulement sanguin.

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A D D E N D U M

A HISTOCHEMICAL SURVEY OF THE ADRENERGIC AND CHOLINERGIC
NERVES IN THE WING VESSELS OF PTEROPUS GIGANTEUS
AND THE EFFECTS OF DENERVATION

The elaboration of specific and highly sensitive fluorescent methods for the histochemical determination of bio-amines (Falck 1962; Falck et al, 1962; Falck and Owman 1965; Corrodi and Jonsson 1967) has led to a more detailed understanding of the role of the innervation in different blood vessels. The fluorescence pattern derived from such techniques gives an overall view of the distribution and density of the innervation, which may later be followed by a closer examination of the nerve-muscle relationship at the electron microscope level (Speden 1970).

Adrenergic nerves form a dense network in their terminal regions. This has been described by Hillarp as 'the autonomic ground plexus' (1946; 1959) from which the neurotransmitter is released (Norberg 1967; Van Orden et al, 1966). A typical autonomic plexus in the wall of muscular blood vessels consists of a two-dimensional network of varicose nerve terminals lying just outside the media (Falk 1962;

Ehlinger et al, 1966). The density of the plexus is highly variable in both arteries and veins, though these tend to be more dense generally in the corresponding arteries (Norberg and Hamberger 1964; Furness 1971). Fuxe and Sedwall (1965) noted that the number of adrenergic fibres accompanying an artery are directly proportional to the diameter of the vessel and decrease as the size diminishes.

The apparent cholinergic responses to nerve stimulation of peripheral sympathetic pathways in certain vascular beds (Burn and Rand 1960) has raised the question of whether such activities are due to the presence of true cholinergic post ganglionic fibres or of para-sympathetic nerve fibres mixed with the sympathetic nerve supply. This problem has been further complicated recently by the finding that sympathetic (and parasympathetic) sub-systems may contain post ganglionic fibres which are neither adrenergic or cholinergic (Bennett 1972).

The present study has been limited to an investigation of the detailed organization of the adrenergic and cholinergic innervation of the wing vessels by fluorescence histochemistry of the adrenergic transmitter and by histochemistry of cholinesterase, which at present is the only selective technique available for the demonstration of cholinergic fibres at the light microscope level.

With a view, furthermore, to developing a reliable denervation procedure which would allow for later studies on the neurogenic influence on the pulsatile alar veins, the disappearance of the adrenergic transmitter was followed at various time intervals after transection of the brachial

nerve or its metacarpal extensions. Such denervation (fig.), has previously been noted to lead to a loss of tone and of frequency of vasomotion of both intact and perfused metacarpal veins (Peristiany, Lane and Huggel 1969,1971).

M A T E R I A L A N D M E T H O D S

Denervation was carried out in wings of 9 animals anaesthetised with chloroform to facilitate handling and then with nembutal (40 mg/kilo wt.) for deeper sleep under an operation microscope (Zeiss Epitechnoscope). The brachial nerve trunk in one of the two wings was dissected free from the accompanying artery and vein and a section of nerve 1 cm long was removed 3 cm distal to the shoulder joint. The second wing (intact) served as a control for the following investigations.

Tissues were dissected out under chloroform anaesthesia (and the animal subsequently killed) at various time intervals after the denervation: 14 hours, 3 days, 6 days, 3 weeks, 6 weeks. Two entirely non-operated animals were also examined. Two pieces from the neurovascular bundle were taken out, one just proximal to the nerve section and one at the level of the metacarpal bone, from both the intact and denerved wings.

The preparations were immediately frozen in propane-propylene mixture to the temperature of liquid nitrogen and

freeze-dried. Several pieces of artery and vein running on each side of fingers 2 and 3 were then removed from the wings of all 11 animals. The vessels were cut open longitudinally, spread flat on the microscope slides, and dried for at least 1 hour in a dessicator over phosphorus pentoxide.

The freeze-dried neuro-vascular preparations and half of the vascular spreads were exposed to formaldehyde gas (from paraformaldehyde equilibrated in air with 70% humidity) at $+80^{\circ}$ C for 1 hour. The freeze-dried material was then embedded in paraffin in vacuo and sectioned serially in the transverse plane at 6μ thickness and mounted in Entellan (Merck). The spreads were also mounted in Entellan for fluorescent microscopy. For further technical details, see Bjorklund, Falk and Owman (1972). The vascular spreads not treated in formaldehyde were pre-incubated with Mipafox (N,N -di-iso-propyl-phosphorodiamide fluoride, 4×10^{-6} M) followed by incubation for 4-24 hours in acetylthiocholine to demonstrate acetylcholinesterase (Holmstedt 1957, Koelle 1963). Eosin was used as counterstain - the slides were mounted with Entellan.

RESULTS AND COMMENTS

- a) Distribution of adrenergic and cholinesterasic activity in the normal preparation:

Adrenergic nerves, brought to light by fluorescence microscopy after formaldehyde condensation, formed a very dense plexus of fibres in the adventitia of the arteries (fig. 1 A), and they often had well visible varicosities interspersed at regular intervals along the fibres (fig.1 B) No fluorescent nerves were seen to penetrate into the arterial medial layer (fig. 1 C) and, hence, the nerve plexus can be considered as essentially two dimensional, enclosing the arterial musculature. The main arteries were supplied with adrenergic nerves to the same extent both at the brachial and the metacarpal levels - also smaller arterial branches received numerous fluorescent fibres (fig. 3 C).

Whole-mounts of the alar veins revealed an adrenergic nerve plexus consisting of varicosed fibres but having a much lower density than that of the arteries (fig. 2 A). Many of the fibres formed a distinct layer on the surface of the medial layer in the vessel, and several others penetrated into the muscle layer to run irregularly among the smooth muscle cells (fig. 3c).

The nerve trunk ran between, and close to, the corresponding artery and vein. It consisted of 10-15 bundles of myelinated fibres which were well visible in the fluorescence microscope owing to their slight background fluorescence (fig. 3C). Fibres with a specific (formaldehyde-

induced) fluorescence could, however, not be distinguished within the nerve fascicles. The arrangement was similar at both brachial and digital levels.

In the walls of both the wing arteries (fig. 3A) and veins (fig. 3B), fibres demonstrable by cholinesterase technique formed plexuses with the same density and distribution as the adrenergic ones visualized by the formaldehyde method (fig. 1A and 2A). However, cholinesterase staining was relatively faint also after prolonged incubations.

- b) The effects of denervation on the distribution of adrenergic and cholinesterase activity:

The outcome of the denervation experiments (unilateral transection of the brachial nerve) was best studied in the vascular whole mounts, where a complete picture was obtained of the nerve plexus in the full circumference of the entire vessel preparation. The relative extent of denervation was always similar in the arteries and in the veins. In some preparations taken 14 hours after the nerve section, the number of fluorescent nerves was the same as in the non-operated control wing, but the fluorescent intensity was often reduced (fig. 2B). Other preparations taken after the same postoperative interval showed a clear reduction also in the number of fluorescent fibres. Three days postoperatively, however, the reduction in the number of visible adrenergic fibres was a constant phenomenon (fig. 1B and 2C). Characteristically, longitudinal "streets" of varying width and entirely devoid of fluorescent fibres alternated with streaks in which the nerve plexus was well visible, although the

fluorescence in the remaining fibres always had a lower intensity than normally.

The observations suggest the following possible explanation: shortly (14 hours) after the nerve section, the transmitter has not yet leaked out completely from the severed adrenergic fibres, and therefore, an amine fluorescence is still visible in all the nerves, though reduced in intensity in many, giving the impression of an overall reduction in fluorescence intensity in the whole plexus. From 3 days onwards, the fluorescence picture was the same, indicating that the amine fluorescence had now disappeared from all severed nerves. In some parts of the plexus, the nerve strands consist entirely of severed fibres which correspond to those above-mentioned areas which are entirely devoid of fluorescent nerves. In other parts, severed nerves run together with intact nerves in the strands, and therefore contribute only to a reduction in the fluorescence intensity of the meshes in such areas. The arrangement of the remaining nerves after nerve section offered a possibility to estimate the degree of denervation:

The plexus that could be visualized (although the fluorescence intensity was reduced) occupied 30-50 per cent of the circumference of the vessel, which would mean that considerably less than this amount of individual intact nerves remained (because the visible plexuses had most probably also lost some of their fibres in the strands). The degree of denervation was not higher in the vessels removed 6 days, or 3 weeks (fig. 1D and 2D) or 6 weeks after the transection.

The extent of cholinergic denervation of the vessels after transection of the brachial nerve was similar to that described above with regard to the adrenergic nerve plexuses.

Parrafin sections from the freeze-dried neuro-vascular preparations taken proximal to the nerve lesion revealed the presence of numerous intensely fluorescent "dots" and small areas, which were either spread uniformly (fig. 3D) or collected in groups (fig. 3E) within the brachial nerve. This phenomenon, which was due to an accumulation of the fluorophore resulting from piling-up of the adrenergic transmitter proximal to the nerve section, was most clearly brought into evidence within the first postoperative week. The amine accumulation was also visible at 3 weeks after the nerve sectioning, but the fluorescent nerves had disappeared 6 weeks after the operation. Accumulation of the amine fluorescence could not be observed in those nerves enclosing the blood vessels.

D I S C U S S I O N

The problem of a neural influence on the pulsatile activity of the veins in the wings of Chiroptera has long been a matter of controversy. This has mainly been due to the lack of morphological evidence for the presence of nerves in the vessels, though partly also, to the inability to elicit a response to local nerve stimulation (see e.g. Nicoll 1964). In 1966, Mislin demonstrated that nerve fibres did in fact cover the veins in digital regions of the bat wing. Since then, the presence of an adrenergic nerve plexus (Lane, Schönenberger and Huggel 1971), and of Cholinergic nerve fibres (Lane and Huggel 1969; Lane 1972), have been reported in the alar veins of *Rousettus aegyptiacus*.

The artery and vein, both at the brachial and metacarpal levels, in the wings of *Pteropus giganteus* were found in the present study to receive a prominent supply of both adrenergic and cholinesterase-containing nerve fibres, in agreement with previously reported preliminary observations (Schönenberger and Lane 1971). In the alar arteries, the nerve plexus was very dense and limited to an almost two-dimensional system in the adventitia, superimposed on the media, which was thus devoid of nerves in its deeper layers. This pattern follows the same general pattern of arterial innervation reported from fluorescence-microscopic studies of various structures in other mammals (e.g. Ehlinger, Falk and Sporrang 1966; Falk and Owman 1966; Lever, Spriggs and

Graham 1968; Schenk and El Badawi 1968; Furness 1971). It appears that only a few of the arterial muscle cells are under direct neural influence, and that the impulses leading to a generalized contraction in the medial layer are propagated electronically between the non-innervated (predominating) individual smooth muscle cells. The alar veins received a more wide-meshed adrenergic network of nerve fibres than the arteries, and they formed a three-dimensional system partly on the surface of the media and partly supplying the underlying muscle layer by branches running in all directions between the smooth muscle cells. This agrees with the general experience that the arterial vessel has a more pronounced adrenergic innervation than the accompanying vein (Furness 1971).

Both the arteries and veins in the wing of Pteropus giganteus also received cholinesterase-containing nerves to the same extent as the adrenergic fibres. Although the type of cholinesterases present in Chiroptera are not identified, the inhibitor (Mipafox) utilized in the present study is usually applied for the demonstration of specific acetylcholinesterase (Holmstedt 1957; Koelle 1963). Histochemical demonstration of acetylcholinesterase is at present the only selective light-microscopic technique available for selective visualization of cholinergic nerves. The activity of cholinesterase (defined as specific acetylcholinesterase on the basis of the inhibitor used) was fairly low in the vascular nerves as indicated by the prolonged incubation time needed to obtain rather moderate staining intensity. This is a feature that has also been noted for certain sensory nerve fibres (Ehlinger 1966) as opposed to parasympathetic nerves, and it may have two implications: either

that the presently described cholinergic fibres indeed are parasympathetic but have an unusually low cholinesterase activity, or that they are sensory. It appears to be a general impression from studies on a variety of vascular areas that the supply of cholinesterase-containing fibres (at least in arteries and arterioles) is qualitatively similar to and as widespread as the adrenergic innervation (Schenk and El Badawi 1968). However, it can not be considered fully established that the enzyme activity demonstrated by these investigators represents specific cholinesterase - if it indeed does, it is still not known whether the cholinergic vascular nerves in question were parasympathetic or sensory. In an electron microscopic study combined with fluorescence and cholinesterase histochemistry and denervation experiments it was shown that sympathetic nerves ran together with parasympathetic fibres in the same strands of a common autonomic ground plexus in the walls of pial arteries (Edvinsson, Nielsen, Owman and Sporrang 1972). However, it is at present not possible to decide whether the cholinergic systems in the alar arteries and veins of Pteropus are parasympathetic or sensory or perhaps even a mixture of the two types: the distinction requires electron microscopy. The presence of cholinergic motor nerves is supported by the observations that topical application of acetylcholine has a chronotropic influence on the pulsatile alar veins, the effect on the frequency, moreover, being biphasic depending on the dose supplied (see Lane 1972).

Denervation at the metacarpal level is associated with a reduction of tone, frequency and amplitude of vasomotion. A similar result is obtained when the vein is perfused to

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free it from contiguity with the arterial pole (fig.). The present study has demonstrated, however, that even sectioning of the brachial nerve trunk only results in a partial denervation. The most reasonable explanation for this seems to be that a considerable portion of the vascular nerve plexi, both at the brachial and metacarpal levels, arise from preterminal nerves that enter the wings with the vessels and continue within the vessel walls rather than in the brachial nerve or its extensions. In accordance with this assumption, accumulation of fluorogenic transmitter did not appear to take place in the vascular adrenergic nerves following brachial transection. Since it has now been well established that the alar vessels of Chiroptera are innervated, a subsequent study on the physiological role of these nerves will require, among other things, a reliable technique for complete denervation.

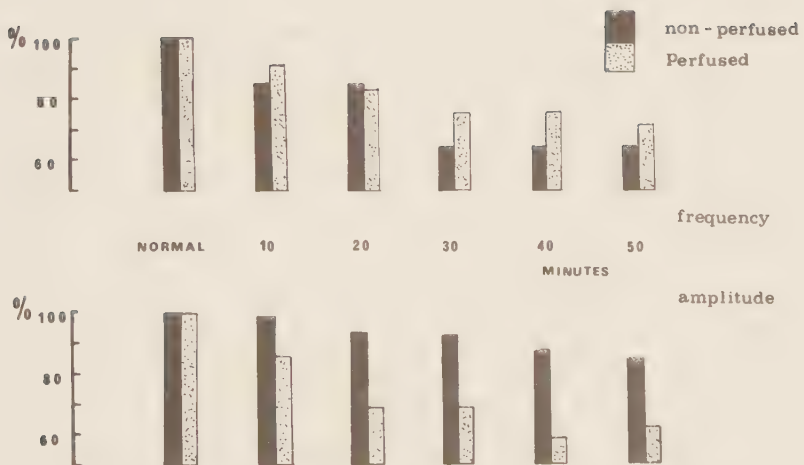


Fig. The effect of denervation, at the proximal end of the metacarpal nerve bundle, on frequency and amplitude of vasomotion of perfused and non-perfused wing digital veins of a Megachiroptera. The lesser reduction in amplitude of the non-perfused preparation could be attributed to the continuity with the arterial pole, whose flow pattern may also be altered following denervation.

S U M M A R Y

The neuro-vascular complex was investigated at the brachial and digital levels in the wings of Pteropus giganteus (Megachiroptera). Fluorescence histochemistry of the adrenergic transmitter using the formaldehyde method revealed a dense plexus of adrenergic nerve terminals in the adventitia of the main arteries. No fibres penetrated into the muscular media. The pulsating veins received a less well-developed fluorescent plexus which, however, was distributed throughout the muscular wall. The vessels also received cholinergic nerves, demonstrated by the cholinesterase method, whose number and distribution resembled the adrenergic plexus in both the artery and vein, respectively. Transection of the brachial nerve resulted in a pronounced, though not complete, adrenergic and cholinergic denervation of the vessels examined at the metacarpal level 14 hours to 6 weeks postoperatively. The outcome of the denervation experiments is probably related to the finding that the adrenergic (and perhaps also the cholinergic) vascular nerves enter the wing not only in the brachial nerve trunk but also together with the blood vessels.

LEGENDS TO ILLUSTRATIONS

Fig. 1. Fluorescence photomicrographs of whole-mounts of Pteropus wing artery (metacarpal level). The vessel has been cut open and mounted flat, the longitudinal axis being oriented horizontally in the pictures. (A) Dense plexus of adrenergic nerves in artery from non-operated wing. X 110. (B) Three days after transection of the brachial nerve there has been a reduction in the density of the arterial adrenergic nerve plexus, facilitating the demonstration of the beaded appearance of the fibres. X 450. (C) Denervation 6 days: Longitudinal streaks of fluorescent adrenergic nerves alternating with completely denervated areas in the vessel wall. X 110. (D) Example of wing artery 3 weeks after brachial nerve transection: Longitudinal streaks of adrenergic nerves remain to approximately the same extent as in C X 110.

Fig. 2. The adrenergic nerve plexus in the metacarpal wing vein at the same level as the arteries illustrated in Fig. 1. Orientation of the whole-mounts as in Fig. 1. (A) The adrenergic plexus in the vein is less dense than in the corresponding artery (ch. Fig. 1A) X 160. (B) Wing vein 14 hours after brachial nerve transection. The vessel has been prepared, photographed and reproduced in identical manner as the vessel from the non-denervated control wing in A. Note that the plexus after nerve section has the same density, but lower

fluorescence intensity, compared to the control. X 110. (C) Example of metacarpal vein 3 days after nerve transection: several fluorescent nerves remain in the vessel wall. X 160. (D) Three weeks after denervation: note groups of fluorescent nerves remaining in otherwise empty vessel wall. X 110.

Fig. 3. (A) Acetylcholinesterase in a dense plexus of nerves arranged as the adrenergic nerve plexus (cf. Fig. 1 A). The longitudinal axis of the vessel (whole-mount) is oriented vertically. X 150. (B) Acetylcholinesterase in the corresponding metacarpal vein forming a plexus resembling that of fluorescent fibres (cf. Fig. 2 A). The longitudinal axis of the vessel (whole-mount) is oriented vertically. X 110. (C) Transverse section of freeze-dried neuro-vascular complex at the metacarpal level from a non-operated wing. The main artery (above) is enclosed by a dense fluorescent nerve plexus that does not penetrate into the muscular media: note autofluorescent internal elastic membrane. Pulsatile vein (below) with adrenergic nerves present at all levels throughout the muscle layer. Between the vessels is the nerve bundles, visible through its slight background fluorescence. Note heavily innervated small artery (right) and vein (left). X 95. - (D) Transverse section of brachial nerve just proximal to division (performed 6 days before). Fluorescent dots reflect accumulation of transmitter in diffusely distributed adrenergic nerves. X 70. (E) Another example of transmitter accumulation proximal to nerve lesion (performed 14 hours before): note that the fluorescent fibres in the brachial nerve of this animal are oriented peripherally in the trunk. X 70.



Fig 1



Fig. 3

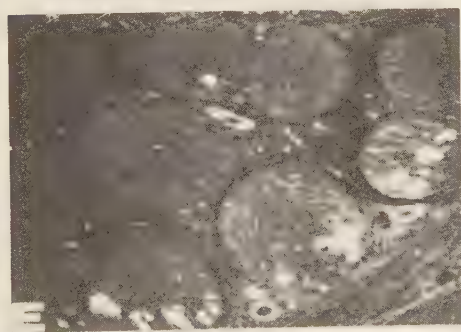
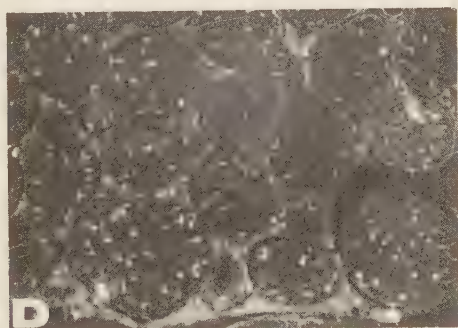


Fig 3

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